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Nasal protein profiles in work-related asthma caused by different exposures

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Abstract

Background The mechanisms of work-related asthma are incompletely delineated. Nasal cell samples may be informative about processes in the lower airways. Our aim was to determine the nasal protein expression profiles of work-related asthma caused by different kind of exposures.

Methods We collected nasal brush samples from 82 non-smoking participants, including healthy controls and work-related asthma patients exposed to 1) protein allergens, 2) isocyanates, and 3) welding fumes the day after relevant exposure. The proteome changes in samples were analysed by two-dimensional difference gel electrophoresis and the differentially regulated proteins found were identified by mass spectrometry. Immunological comparison was carried out using Western blot.

Results We detected an average of 2500 spots per protein gel. Altogether 228 protein spots were chosen for identification, yielding 77 different proteins. Compared to the controls exposure to protein allergens had the largest effects on the proteome. Hierarchical clustering revealed that protein allergen and isocyanate related asthma had similar profiles, whereas asthma related to welding fumes differed. The highly overrepresented functional categories in the asthma groups were defence response, protease inhibitor activity, inflammatory and calcium signalling, complement activation, and cellular response to oxidative stress. Immunological analysis confirmed the found abundance differences in Galectin 10 and Protein S100-A9 between the groups.

Conclusions Work-related asthma patients exposed to protein allergens and isocyanates elicit similar nasal proteome responses and the profiles of welders and healthy controls were alike. Revealed biological activities of the protein expression changes are associated with allergic inflammation and asthma.

Introduction

Occupational exposure can initiate or trigger asthma, leading to the development of different types of work-related asthma (WRA) (1). Protein allergens at workplaces cause asthma via Immunoglobulin E (IgE)-associated mechanisms, which are similar to allergic asthma unrelated to work (2). Asthma associated with isocyanate exposure is often IgE-independent, but the mechanisms are incompletely delineated. Welding fumes and aerosols composed of potentially hazardous metals and gases are also common exposures among WRA patients (3, 4). These fumes diminish functionality of local and circulating immune cells but the mechanisms in airway diseases are poorly understood (5).

Asthma and rhinitis commonly coexist and this is also true in WRA. Up to 90% of WRA patients report work-related rhinitis symptoms (6). Nasal epithelium provides an important physico-chemical and immunological barrier against the different factors targeting lower airways, but the mechanisms explaining the correlations between these diseases are not fully understood. According to the united airway concept, both diseases could be secondary to the same disease mechanisms occurring throughout the respiratory tract (7).

Proteomic approaches offer great potential for the systematic analysis of complex biological airway samples and have the advantage of assessing the presence and abundance of gene products (i.e., proteins), which are functionally relevant to the clinical phenotypes of airway diseases (8). A nasal brush sample (NBS) is a relatively non-invasive specimen containing proteins secreted from epithelial and inflammatory cells (9). Induced sputum and nasal lavage fluid (NLF), have been applied to characterize proteomic changes in airway diseases (10, 11). The identified proteins in these samples have been mostly identical (12) suggesting that also NBS may be useful in the research of upper airway epithelia and as a surrogate of lower airways.

We assessed the NBS proteome in patients with WRA who were exposed to protein allergens, isocyanates or welding fumes, and in healthy subjects. Our aim was to determine whether the proteomics method could reveal differences between the protein expressions of these groups and to investigate potential reaction mechanisms. Our premise was that nasal samples could reflect the protein profile of the entire airways.

Materials and methods

Study design and population

The study population included WRA patients examined at the Finnish Institute of Occupational Health during 2009-2012 (Figure 1). Their asthma diagnosis was confirmed by demonstrating reversible airway obstruction or bronchial hyperresponsiveness. They were exposed at work to 1) protein allergens, 2) isocyanates, or 3) welding fumes. Their asthma symptoms emerged while they were exposed to the occupational agent, and worsened during exposure. The control group comprised healthy men who carried out their military service 1986-1990 and participated in a follow-up study in 2009 (13). Current smokers were excluded. A subset of samples were used in the proteomic analysis and few found differences were compared both in the whole population and in those excluded from proteomic analysis. In order to analyse representative and homogeneous groups, NBS from eight participants in each group were selected based on clinical symptoms to represent their groups, outliers were not included. The clinical characteristics of these groups are presented in Table S1. The study was

approved by the ethics committee of Helsinki University Central Hospital (approval number 284/13/03/00/08, 125/13/03/00/09). All participants signed their informed consent.

Methods of clinical tests, controlled exposure to occupational agent as well as induced sputum and nasal brush sample preparation are described in Supplementary material 1.

Two-dimensional differential gel electrophoresis and gel spot protein identification

For proteomics analysis, 2 ml of NBS supernatant was concentrated. Protein samples were prepared and two-dimensional differential gel electrophoresis (2D-DIGE) was performed, as previously described (12). For protein identification, in-gel digestion was conducted for the chosen gel spots and the resulting peptides were extracted (12) and dried in a vacuum centrifuge. Each peptide mixture was analysed by an automated EASY nanoLC 1000 (Proxeon, Thermo Fisher Scientific Inc., USA) coupled to an electrospray ionization quadrupole orpitrap mass spectrometer (QExactive, Thermo Fisher Scientific Inc., USA). Reverse-phase separation of peptides was carried out using a 75 µm × 15 cm Acclaim PepMap100 C18 column (Dionex, Thermo Fisher Scientific Inc., USA) at a flow rate of 300 nL /min. Peptides were eluted from the column with a linear gradient of 5–35% solvent B (0.1% formic acid in 95% acetonitrile) in 80 minutes. Solvent A was 0.1% formic acid in 5% acetonitrile. We searched for the mass fragment spectra obtained in the SwissProt database (www.uniprot.org) against human entries using Proteome Discoverer 1.4 (Thermo Fisher Scientific Inc., USA).

Immunological comparison

Abundances of Glutathione S-transferase 1 (GSTP1), Galectin 10 (LEG10), Protein S100-A9 (S10A9), and Calcyphosin (CAYP1) were compared by Western blotting in the whole study population using precast 26-well 12% Criterion TGX gels (BioRad), as previously described (12). The gel lane was loaded with 10 µl of untreated NBS. Primary antibody dilutions were 1:100 for S10A9 (Abcam 24111), 1:10000 for LEG10 (Abcam 157475), 1:2000 for GSTP1 (Abcam 47709) and 1:4400 for CAYP1 (Abcam 188470). Immunoblots were stained with anti-rabbit or anti-mouse peroxidase-conjugated immunoglobulins (1:2000) (Dako Cytomaton) and chemiluminescent HRP-substrate ECL detection reagent (Perkin Elmer). They were visualized by an Image Quant LAS 4000 mini quantitative imager (GE Healthcare Biosciences). ImageQuant TL (GE Healthcare Biosciences) was used to calculate the intensities of the protein bands, which were normalized to the band intensity of a pool containing all samples.

Statistical and bioinformatical analyses

Continuous variables were expressed as means (± standard deviation) or median (interquartile range) depending on their distribution and categorical values as percentages. Logaritmic transformation was used to attain normal distribution of continuous variables. The differences between the groups were analysed using Student's *t*-test or Anova and Fisher's LSD test for post hoc comparisons. Mann-Whitney U-test or Kruskal-Wallis test or was used when normal distribution was not attained after logarithmic transformation, and Chi-square test for categorical values. We computed Spearman´s correlation between continuous values. A p-value of <0.05 was considered statistically

significant. IBM SPSS Statistics for Windows, Version 20.0 (Armonk, NY: IBM Corp.) software was used for analysing the clinical parameters and for correlations. Principal component analysis (PCA) and hierarchical clustering of the identified differentially abundant proteins were performed with DeCyder Extended Data Analysis software (Version 7.0, GE Healthcare), using average linkage and the Euclidean metric as a distance measure. Western blot intensities were statistically analysed using GraphPad Prism 5 software (GraphPad Software).

Ingenuity Pathway Analysis (IPA) (Qiagen), String (string-db.org) and Enrichr (amp.pharm.mssm.edu/Enrichr) were used to investigate interactions, functions and pathways of relevance in the identified proteins.

Results

Study population

Table 1 presents the characteristics of 82 participants. Work-exacerbated rhinitis symptoms were reported by 27 (93%) of the WRA patients in the protein allergen group, 12 (100%) in the isocyanate group, and 11 (79%) in the welding group (p= 0.139). Nonspecific bronchial hyperresponsiveness was detected in 17 (59%), 4 (33%), 7 (54%) of the patients of the above groups, respectively (p=0.333) (n=54). Nasal steroid was withdrawn at least three days before sample collection; 12 (22%) of the WRA patients had used a nasal steroid during the previous four weeks. Positive skin prick tests (SPT) to common environmental allergens were detected more often in the protein allergen group (82%) than in the isocyanate (42%), welding (43%) or control (30%) groups (Table 1). Positive SPT to the occupational allergen of controlled exposure was found in 26 of 27 tested participants in the protein allergen group, but none in the isocyanate or welding group reacted to the isocyanate-albumin conjugate or metal suspension. Similarly, specific IgE to the occupational allergen was detected in 21 of 28 tested participants in the protein allergen group, but in none of the 10 tested in the isocyanate group. Fractional exhaled nitric oxide (FeNO) was highest in the protein allergen group, whereas nasal eosinophil count was biggest in the isocyanate group.

Two-dimensional differential gel electrophoresis (2D-DIGE) differences in protein abundances

DeCyder software matched on average 2500 spots per DIGE gel, no significant difference was observed between the total number of protein spots of the study groups. 228 protein spots with changed intensity from statistical analysis (Student's t –test <0.05, a fold change of ≤ -1.5 or ≥ 1.5) between groups were identified (Table S2, Figure S1) revealing altogether 77 different proteins (Table 2), as several proteins were identified from multiple gel spots. As many as 95% of the proteins found are known to have an extracellular location (Gene Ontology (GO):0070062, GO:0005615). In addition to plasma-derived proteins, various nasal epithelium proteins were detectable. Of the identified molecules, 32 were involved in inflammatory responses, 36 in cellular movement, 20 in free radical scavenging, 42 in cell death and survival, and 18 in allergy (Table S3). Calcium binding proteins were also enriched among the proteins identified. Three up-regulated and seven down-regulated proteins were common to all the WRA groups.

Clustering analyses revealed similar protein regulation patterns in protein allergen and isocyanate groups

Unsupervised classification analyses were applied to the data to obtain an overview of protein abundance patterns. Hierarchical clustering analysis (Figure 2A), in which proteins with similar manifestation profiles are clustered together, showed that the protein patterns from participants with protein and isocyanate WRA resembled each other, and that the patterns in the welding group differed from the other WRA groups. When PCA was applied to the set of all the proteins identified in the nasal epithelium, the groups were positioned into separate quadrants, implying that different types of occupational exposures appear to perturb the global variability of nasal epithelial protein levels (Figure 2B). The PCA of identified protein spots (Figure 2C) showed clear separation of the spots to two abundance clusters, similarly to the abundance differences in Figure 2A.

Group-specific differently abundant proteins

The largest effects on the proteome of nasal epithelium were in the protein allergen group (Tables 2 and S1, Figures 2A and S2), in which 20 proteins were up-regulated and 43 down-regulated in comparison to the healthy controls. Exposure to isocyanates showed changes in 32 proteins and welding in 19 proteins. When WRA groups were compared with each other, the largest proteome differences observed were between the protein allergen and welding groups (45 different proteins). Welding-specific changes were decreased levels of heat shock cognate 71 kDa protein spots, and increased amounts of pyruvate kinase isozymes M1/M2 and Fatty acid binding protein.

Protein S100-A9 abundance was increased in all WRA groups, but protein S100-A8 (S10A8) was identified only for the protein allergen group. Other calcium binding proteins, calcyphosin (CAYP1) and annexins A1 and A2 (ANXA1, 2) had decreased protein levels in the protein allergen and isocyanate groups. The protein allergen group seemed to also contain low levels of annexins 3 and 5.

We identified several proteins involved in the detoxification of reactive oxygen species (Table 2). The catalase level was increased, whereas the levels of glutathione (GSH) synthesis-linked proteins s-formylglutathione hydrolase, glutathione synthetase and adenosylhomocysteinase were diminished, as was the GSH conjugation catalysing enzyme, GSTP1. Peroxiredoxins 1, 2 and 5 (PRDXs) levels were lower in the protein allergen group, and superoxide dismutase (SODC) levels decreased in the protein allergen and isocyanate groups. Changes in the abundancies of thioredoxin and protein disulphide-isomerase, which also belongs to the thioredoxin superfamily, were specific to isocyanate exposure.

Pathways and networks of proteins

Figure S2 shows the protein association networks of the proteins identified in each WRA group. Mitogen-activated protein kinases (MAPKs) and nuclear factor kappa B (NFκB)-signalling pathways consistently emerged in network searches, including single protein fishes, and were thus added to Figure S3 networks.

Pathways and diseases linked to the observed proteomic changes of the nasal mucosa in the IPA search are presented in Table 3. The reported ethanol degradation pathway, common to the protein allergen and isocyanate groups, is most likely connected to the aldehyde dehydrogenases involved in the metabolism of corticosteroids. The nuclear factor erythroid 2-related, factor 2 (Nrf2)-mediated oxidative stress response pathway is common to protein allergen and isocyanate WRA groups. Nrf2 is a key transcription factor that regulates antioxidant defence in macrophages and epithelial cells (14), and is a component of the GSTP1 interactome (15). Protein-allergen and

Isocyanate exposed asthma patients experienced more severe nasal symptoms and had a suggestive increase in nasal and sputum eosinophils which may be linked to inflammatory response in Table 3.

Immunological comparison of nasal brush samples

Several of the identified proteins are associated with allergic reaction mechanisms, among them GSTP1, LEG10 and S100A9, which were selected for further analysis as high quality commercial antibodies were also available for them. The unfamiliar fourth analyte, calcium binding protein CAYP1, may play a role in cellular signaling events..The Western blot analysis revealed similar abundance differences in a few proteins in the whole study population (Figure 3) and in 50 subjects who were excluded from proteomic analysis (Table S4). The levels of the calcium binding protein S10A9 and T cell proliferation suppressor protein LEG10 were up-regulated in all asthma groups. The oxidative stress defence protein GSTP1 showed a trend of reduction in the protein allergen group in comparison to the control group, whereas there was tendency for decreased levels of CAYP1 in all WRA groups compared to the controls. Abundances of the GSTP1 and CAYP1 proteoforms might vary in opposite directions, and together with the statistically relatively small study population, they could be the underlying causes for the lack of clear group differences.

Associations between protein abundances and clinical parameters

Protein abundances of GSTP1, LEG10, S10A9 or CAYP1 did not correlate with age, sputum eosinophils, FeNO, nNO or total IgE, whereas moderate correlation was seen between LEG10 and blood eosinophils ($r=0.536$, $p=0.008$) as well as S10A9 and FEV_1/FVC ($r=0.438$, $p=0.017$). GSTP1 was higher in men ($p=0.016$), but no significant gender difference was found in Western blot intensities of GSTP1 ($p=0.242$) or other protein abundances.

Discussion

Proteomic analysis of NBS is a relatively non-invasive way to evaluate airway inflammation. Hierarchical clustering analysis of the protein abundance patterns of NBS indicated that the nasal protein profile in protein-allergen- and isocyanate-related asthma groups are similar, and differ from those of healthy controls, whereas the welding group pattern bore more resemblance to that of the controls.

Clinical tests targeted to show causal relationship with asthma and occupational exposure (e.g. specific IgE, specific inhalation challenge) may select patients based on disease mechanisms. To avoid this preselection, we studied WRA patients. The vast majority of our WRA patients had concurrent work-aggravated rhinitis symptoms, as others have reported (6). The use of nasal steroids among the three WRA groups ($p=0.75$) during the four previous weeks did not significantly differ, suggesting that steroids had no significant effect on the protein abundances of these groups. Highest FeNO level reflecting Th2-derived inflammation in protein-allergen-related asthma group was similar to pervious findings (16). Isocyanate-related asthma patients had more nasal, but not sputum, eosinophils than other groups. Raulf-Heimsoth et al. detected an increase in sputum eosinophils but not in NLF after a positive isocyanate challenge (17).

Few studies have reported the proteome of work-related airway diseases. Mörtstedt et al. performed targeted proteomic analyses of NLF before and after a persulfate challenge among hairdressers with bleaching powder-associated rhinitis as compared to healthy hairdressers and atopic subjects (18). They detected changes in proteins related to inflammatory responses and oxidative stress, but the changes were not specific to rhinitis patients. Studies of nasal proteome in seasonal allergic rhinitis have shown a perennial inflammatory response in nasal mucus and lack of adequate reaction to allergens in season (19-21).

Proteomic changes in airway diseases have been characterized from induced sputum and NLF (10, 11). The most abundant proteins observed in these biofluids are plasma proteins, which are presumably derived from diffusion across the blood-air barrier (e.g. albumin, transferrins, immunoglobulins, alpha-1-antitrypsin), and interfere with the detection of less abundant proteins (12). NBS has rarely been used to investigate the proteome of patients with respiratory disorders (22). In this study, NBS provided good quality material, which was not distracted by blood derived high abundance proteins.

Protein abundance patterns analysed by hierarchical clustering were similar in protein allergen- and isocyanate-related WRA. IgE-mediated sensitization to isocyanates was not detected, while it was seen to occupational protein allergens in all but one case. This suggests that protein-allergen- and isocyanate-related WRA share non-IgE mediated immunological mechanisms. The welders' profile differed from that of the other WRA groups and controls, proposing different underlying mechanisms.

Our results indicate that the nasal epithelial proteome of the WRA patients is highly enriched in processes related to inflammatory and calcium signalling, free radical scavenging and oxidative stress response, and metabolism. The most relevant signalling networks were through the pathways associated with redox sensitive transcription factors, NFκB and Nfr2, and with MAPKs, which differentially regulate pro-inflammatory cytokine genes and protective antioxidant genes (23-25). Nrf2-deficient mice have a heightened susceptibility to asthma, including elevated oxidative stress, inflammation, mucus, and airway hyperresponsiveness (26). Airway inflammation and remodelling in asthma involves degradation of the extracellular matrix. Among the identified proteases and their inhibitors was Serpin B3, which inhibits inflammation and promotes epithelial proliferation with increased transforming growth factor-beta secretion (27). Several inflammatory defence proteins with both increased and decreased abundance were detected in subjects exposed to protein allergens and isocyanates.

LEG10 (aka Charcot-Leyden Crystal (CLC) protein, Eosinophil lysophospholipase) has been associated with eosinophilic inflammation in allergic diseases (28, 29) and with the function of regulatory T-cells (30). LEG10 is mainly released from eosinophil granules, but it is also expressed by basophils and some T cells (30). It belongs to the galectin superfamily of lectins (31), although its physiologically relevant carbohydrate ligand is unknown. Further knowledge of this would help us understand its role in inflammatory reactions.

Oxidative stress is important for the pathogenesis of lung damage and for the development of lung fibrosis. Among the various enzymatic and non-enzymatic mechanisms that protect cells and tissues from oxidants, GSH, SODs and PRDXs play a key protective role, especially in the lungs (32, 33). A lower level of GSH synthesis enzymes, SODs and PRDXs might induce continuous oxidative stress in the airways. In this study, a decreased abundance of all these proteins was observed in the participants with symptoms on exposure to protein allergens and isocyanates, but not in the welding group, suggesting that the impairment of protection from oxidative stress might play a key role in the pathogenesis of WRA.

GSTP1 plays a significant role in detoxification processes that regulate inflammatory responses stimulated by xenobiotic and oxidative compounds, and is important in determining susceptibility to asthma. GSTP1 conjugates reduce GSH to electrophilic species, and adduct formation with xenobiotics promotes their elimination, whereas binding to protein thiols causes a reversible posttranslational modification, S-glutathionylation, which protects proteins from irreversible oxidations and can modulate their function (34). GSTP1 gene activation is mainly regulated by Nrf2 (35), and the functional association between GSTP and Nrf2 proteins supports the regulatory role of GSTP in the adaption response to cellular stresses produced in the course of inflammatory and oxidative reactions (15). As in the present study, GSTP was decreased in NLF after the persulphate challenge (18).

Ca^{2+} signals are important in inflammatory signalling and in the pathophysiology of airway diseases. Calcium-modulated S100 protein family members S10A9 and S10A8 are both among the most abundant proteins in airway cells during chronic inflammation. They may form a calprotectin complex which can induce cell proliferation, apoptosis, inflammation, collagen synthesis, and cell migration. S10A9 alone mediates fibroblast proliferation, increases mucin production, and is involved in NFκB network and inflammasome activation (36), whereas the protective role of S10A8 in allergic inflammation is to modulate mast cell activation and eosinophil recruitment, and scavenge the oxidants generated by activated leukocytes (37). Synthesis and activation of CAYP1 is induced by the cAMP cascade (protein kinase A) and CAYP1 may regulate cell proliferation and differentiation (38, 39). Interestingly, String DB predicts as functional partners for CAYP1 several MAP kinases, which are activated in response to inflammatory and oxidative stress signals (24). Glucocorticoid regulated annexins interact with cell membrane phospholipids, and are involved in various cellular processes, including endocytosis, exocytosis, membrane-cytoskeletal organization, and migration through the association of partner proteins, including members of the S100 family. ANXA1 is an anti-inflammatory protein that plays a critical regulatory role in the development of asthma (40, 41), whereas ANXA2 promotes fibrinolysis (42). The roles of ANXA3 and 5 in asthma are largely unknown, but their amounts were reduced in the protein allergen exposure groups.

Conclusions

Proteome analysis of NBS provides preliminary results regarding the mechanisms of work-related airway diseases in patients exposed to protein allergens, isocyanates and welding fumes. WRA patients exposed to protein allergens and isocyanates showed similar nasal proteome responses, although the specific IgE was found only among patients exposed to protein allergens. The proteome of patients exposed to welding fumes resembled healthy controls. The identified changes in protein expressions of asthma patients reveal biological activities related to airway inflammation, oxidation reduction, tissue matrix turnover, and inflammatory signalling. Our findings provide new possibilities to biomarker research and the development of diagnostic methods of work-related airway diseases.

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Authors' contributions

All authors participated in conception, design and interpretation of data and approved the final version. HS, AP and IL acquired data, HS and AP analysed data and drafted the article which IL and HW reviewed.

Conflict of interest

The authors declare no conflict of interest.

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Table 1 Characteristics of 82 participants. Continuous variables were expressed as means (± standard deviation) or median (interquartile range) depending on their distribution and categorical values as percentages. Logaritmic transformation was used to attain normal distribution of continuous variables. The differences between the groups were analysed using the ANOVA, Kruskal-Wallis test (when normal distribution was not attained after logarithmic transformation) or Chi-square test.

	Healthy controls (n=27)	Asthma related to protein allergen (n=29)	Asthma related to isocyanate (n=12)	Asthma related to welding (n=14)	p
Age, mean (SD)	43.2 (1.9)	42.7 (10.3)	41.0 (10.9)	43.4 (9.9)	0.880
Sex, male, n (%)	27 (100.0)	10 (34.5)	9 (75.0)	12 (85.7)	<0.001
Duration of work exposure, years, median (Q_1-Q_3)[#]	NA	9.0 (5.0-27.0)	4.0 (2.3-9.5)	15.5 (8.8-25.3)	0.014
Duration of asthma symptoms, years, median (Q_1-Q_3)[¤]	NA	2.0 (2.0-3.5)	1.0 (0.6-2.0)	2.0 (1.8-9.0)	0.006
VAS rhinitis, mm, median (Q_1-Q_3)[#]	12.0 (3.0-25.0)	30.0 (7.5-54.5)	30.0 (2.0-50.0)	10.0 (2.5-45.5)	0.123
VAS nasal congestion, mm, median (Q_1-Q_3)[#]	12.0 (0.0-22.5)	40.0 (14.0-57.3)	50.0 (9.0-80.0)	30.0 (8.5-71.5)	0.003
VAS nasal itching, mm , median (Q_1-Q_3)[#]	1.0 (0.0-11.5)	20.5 (3.3-38.0)	50.0 (2.0-80.0)	14.0 (2.5-45.0)	0.001
Nasal steroid during 4 weeks, n (%)	0 (0.0)	6 (20.7)	2 (16.7)	4 (28.6)	0.053
Inhaled steroid during 4 weeks, n (%)	0.0 (0.0)	17 (58.6)	7 (58.3)	14 (100.0)	<0.001
≥1 SPT positive to common environmental allergen*	8 (29.6)	22 (81.5)	5 (41.7)	6 (42.9)	<0.001
Positive SPT to exposed occupational allergen, n (%)*	NA	26 (96.3)	0 (0.0)	0 (0.0)	<0.001
Total IgE, kU/l, median (Q_1-Q_3)[¤]	34.0 (13.0-73.0)	187 (51.5-309.5)	46.0 (26.3- 70.0)	61.5 (29.3-132.5)	<0.001
Blood eosinophils 10^6/l, mean (SD)	129.3 (65.3)	206.6 (155.0)	148.3 (147.4)	182.5 (130.5)	0.137
FVC % predicted, mean (SD)	96.9 (10.2)	99.4 (10.9)	97.9 (12.4)	95.7 (8.8)	0.706
FEV_1 % predicted, mean (SD)	95.8 (12.7)	91.0 (10.8)	92.9 (7.3)	88.9 (11.5)	0.239
FeNO, ppb, median (Q_1-Q_3)[¤]	13.2 (11.9-16.9)	23.2 (12.8-37.9)	17.0 (10.4-26.9)	23.0 (13.7-32.9)	0.004
nNO, ppb, mean (SD)	836.2 (272.7)	929.5 (351.1)	760.6 (233.8)	896.1 (339.8)	0.400
Sputum eosinophil %, median (Q_1-Q_3), n=57[#]	0.0 (0.0-0.25)	1.0 (0.0-4.0)	1.0 (0.0-1.5)	0.5 (0.0-1.0)	0.044
Nasal eosinophil %, median (Q_1-Q_3)[#]	0.0 (0.0-0.0)	0.0 (0.0-1.0)	1.0 (0.0-1.0)	0.0 (0.0-0.0)	0.009

Kruskal-Wallis test was used; Logaritmic transformation was used; *two participants with negative control wheal ≥2 mm were excluded from analysis; *VAS*, Visual analogue scale of nasal symptoms within a week; *SPT*, skin prick test; *IgE*, Immunoglobulin E; *FVC*, forced vital capacity; FEV_1, Forced expiratory volume in one second; FeNO, exhaled nitric oxide; nNO, nasal nitric oxide.

Table 2 Relationships between differently abundant proteins in study groups. Average ratios (≥|1.5| fold change in abundance) of statistically significant (Student's T-test p-value ≤ 0.05) up- and down-regulated nasal mucosa proteins are presented for the protein allergen, isocyanate and welding exposed asthma groups in comparison to the control group.

Protein Name	UniProt AC	Gene ID	Protein allergen vs Control Av. ratio	Isocyanate vs Control Av. ratio	Welding vs Control Av. ratio
Alpha-2-macroglobulin (A2MG)	P01023	A2M	**1,83**		
Complement C3 (CO3)	P01024	C3	**1,82**		
Complement factor B (CFAB)	P00751	CFB	**1,88**	**1,88**	
Alpha-actinin-4 (ACTN4)	O43707	ACTN4		**-1,77**	**-1,95**
Serum albumin (ALBU)	P02768	ALB	**3,17**	**2,81**	**2,36**
Polymeric immunoglobulin receptor (PIGR)	P01833	PIGR	**1,85**		
Protein-glutamine gamma-glutamyltransferase (TGM3)	Q08188	TGM3	**2**		
Lactotransferrin (TRFL)	P02788	TRFL	**5,43**	**4,95**	**2,87**
Ig mu chain C region (IGHM)	P01871	IGHM	**1,89**		
Serotransferrin (TRFE)	P02787	TF	**1,75**		
Heat shock cognate 71 kDa protein (HSP7C)	P11142	HSPA8			**-2,49**
Protein disulfide-isomerase (PDIA1)	P07237	P4HB		**1,67**	
Histidine ammonia-lyase (HUTH)	P42357	HAL		**1,74**	
Glucose-6-phosphatate isomerase (G6PI)	P06744	GPI			**2,09**
Pyruvate kinase isozymes M1/M2 (KPYM)	P14618	PKM			**2,45**
Ig alpha-1 chain C region (IGHA1)	P01876	IGHA1	**2,04**		
Alpha-1-antitrypsin (A1AT)	P01009	SERPINA1	**2,15**		
Histidine--tRNA ligase; cytoplasmic (SYHC)	P12081	HARS	**-1,57**		
Tryptophan-tRNA ligase; cytoplasmic (SYWC)	P23381	WARS	**-1,67**		
Aldehyde dehydrogenase (AL3A1)	P30838	ALDH3A1	**-2,76**	**-3,4**	**-2,19**
Selenium-binding protein 1 (SBP1)	Q13228	SELENBP1	**-2,05**	**-1,58**	
Glutathione synthetase (GSHB)	P48637	GSS	**-2,08**		
Retinal dehydrogenase 1 (AL1A1)	P00352	ALDH1A1	**-2,25**		
Rab GDP dissociation inhibitor beta (GDIB)	P50395	GDI2	**-2,51**	**-2**	**-1,71**
Glyceraldehyde-3-phosphate dehydrogenase (G3P)	P04406	GAPDH	**-1,51**	**-1,55**	
6-phosphogluconate dehydrogenase (6PGD)	P52209	PGD		**2,32**	**2**
Catalase (CATA)	P04040	CAT	**1,77**	**2,87**	
Zinc-alpha-2-glycoprotein (ZA2G)	P25311	AZGP1		**1,63**	
Serpin B3 (SPB3)	P29508	SERPINB3	**-2,02**	**-1,92**	**-1,72**
26S proteasome non-ATPase regulatory subunit 11 (PSD11)	O00231	PSMD11	**-1,58**		
Haptoglobin (HPT)	P00738	HP	**2,02**		
Isocitrate deheydrogenase [NADP]; cytoplasmic (IDHC)	O75874	IDH1	**-2,74**		
Arginosuccinate synthase (ASSY)	P00966	ASS1	**-2,79**		
Creatine kinase B-type (KCRB)	P12277	CKB	**1,71**		
Fructose-bisphosphate aldolase A (ALDOA)	P04075	ALDOA	**-2,53**		
Leukocyte elastase inhibitor (ILEU)	P30740	SERPINB1	**-1,56**		
GDP-L-fucose synthase (FLC)	Q13630	TSTA3	**-1,97**		
Plasminogen activator inhibitor 2 (PAI2)	P05120	SERPINB2	**-1,8**		
Alcohol dehydrogenase [NADP(+)]	P14550	AK1A1	**-3,06**		

Annexin A1 (ANXA1)	P04083	ANXA1	**-3,78**	**-5,05**	
Annexin A2 (ANXA2)	P07355	ANXA2	**-4,59**	**-5,75**	
Annexin A3 (ANXA3)	P12429	ANXA3	**-1,9**		
Annexin A5 (ANXA5)	P08758	ANXA5	**-2,59**		
S-formylglutathione hydrolase (ESTD)	P10768	ESD	**-2,18**		
Tropomyosin alpha-1 chain (TPM1)	P09493	TMP1	**-1,9**		
Tropomyosin alpha-4 chain (TPM4)	P67936	TMP4			
Actin; cytoplasmic 1 (ACTB)	P60709	ACTB	**-1,68**		
Chloride intracellular channel protein1 (CLIC1)	O00299	CLIC1	**-1,8**		**-1,56**
14-3-3 protein sigma (1433S)	P31947	SFN	**-1,88**		
14-3-3 protein zeta/delta (1433Z)	P63104	YWHAZ	**-2,14**	**-1,58**	**-1,85**
14-3-3 protein epsilon (1433E)	P62258	YWHAE	**-2,63**	**-2,27**	**-1,9**
Serum amyloid P-component (SAMP)	P02743	APCS	**1,75**	**2,01**	
Proteasome subunit alpha type-5 (PSA5)	P28066	PSMA5	**-1,96**		
Kallikrein-7 (KLK7)	P49862	KLK7	**-2,75**	**-2,34**	**-1,89**
Glutathione S-transferase P (GSTP1)	P09211	GSTP1	**-2,6**	**-1,93**	
Complement C1q subcomponent subunit C (C1QC)	P02747	C1QC		**1,6**	
Heat shock protein beta-1 (HSPB1)	P04792	HSPB1	**1,75**	**1,85**	
BPI fold-containing family A member 1 (BPIA1)	Q9NP55	BPIFA1		**-1,96**	
Translationally-controlled tumour protein (TCTP)	P13693	TPT1	**-1,95**	**-1,9**	**-1,59**
Peroxiredoxin-2 (PRDX2)	P32119	PRDX2	**-2,5**		
Peroxiredoxin-1 (PRDX1)	Q06830	PRDX1	**-3,6**		
Phosphatidylethanolamine-binding protein 1 (PEBP1)	P30086	PEBP1	**-2,18**		
Adenine phosphoribosyltransferase (APT)	P07741	APRT	**-3,11**		
Calcyphosin (CAYP1)	Q13938	CAPS	**-4,76**	**-5,15**	
Nucleoside diphosphatate kinase A (NDKA)	P15531	NME1		**-1,58**	
Thioredoxin (THIO)	P10599	THIO		**-2,16**	
Superoxide dismutase [Cu-Zn] (SODC)	P00441	SOD1	**-1,98**	**-2,21**	
Cofilin-1 (COF1)	P23528	CFL1	**-2,13**	**-2,13**	
Nucleoside diphosphatate kinase B (NDKB)	P22392	NME2	**-2,26**		
Prolactin-inducible protein (PIP)	P12273	PIP	**2,22**		
Peroxiredoxin 5 (PRDX5)	P30044	PRDX5	**-2,85**		
Peptidyl-prolyl cis-trans isomerase A (PPIA)	P62937	PPIA	**-2,86**		
Fatty acid binding protein, epidermal (FABP5)	Q01469	FABP5			**2,69**
Galectin 10 (LEG10)	Q05315	CLC	**4,04**		
Haemoglobin subunit beta (HBB)	P68871	HBB			**-2,27**
Protein S100-A9 (S10A9)	P06702	S100A9	**2,2**	**7,11**	**8,71**
Protein S100-A8 (S10A8)	P05109	S100A8	**2,43**		

Table 3 Pathways and diseases linked to the observed proteomic changes in the nasal mucosa after exposure to protein allergen, isocyanate and welding fume.

	Canonical pathways	
Protein allergen	Isocyanate	Welding
Acute Phase Response Signalling	Superoxide Radicals Degradation	Glycolysis I

NRF2-mediated Oxidative Stress Response	NRF2-mediated Oxidative Stress Response	Cell Cycle: DNA Damage Checkpoint Regulation
Tryptophan Degradation X	Ethanol Degradation IV	Myc Mediated Apoptosis Signalling
LXR/RXR Activation	Aryl Hydrocarbon Receptor Signalling	ERK5 Signalling
Ethanol Degradation IV	Complement System	HIPPO signalling

	Diseases and Disorders	
Protein allergen	Isocyanate	Welding
Inflammatory Response	Inflammatory Response	Dermatological Diseases and Conditions
Immunological Disease	Dermatological Diseases and Conditions	Immunological Disease
Dermatological Diseases and Conditions	Immunological Disease	Neurological Disease
Haematological Disease	Neurological Disease	Psychological Disorders
Inflammatory Disease	Skeletal and Muscular Disorders	Cancer

The top five canonical pathways and disease-associated functions from the Ingenuity Pathway Analysis of the affected protein levels in protein allergen, isocyanate and welding challenged groups compared to the control group. The p-values of likelihoods for the protein list of each study group were calculated using the Fisher Exact test. The top five p-values were for protein allergens from E-21 to E-08, for isocyanate from E-11 to E-07 and for the welding group with the shortest identified protein list from E-11 to E-05.

Figure legends

Figure 1 Study flow chart. HDI, Hexamethylene diisocyanate; MDI, Methylene diphenyl diisocyanate.

Figure 2 Clustering analyses of the differentially abundant proteins between study groups. A heat map of hierarchical clustering (A), on which nasal epithelial proteins with decreased abundance are marked in green and those with increased abundance in red; and Principal Component Analysis (B-C), in which spheres correspond to a study group (Fig. 2B) or to an identified gel spot (Fig. 2C). In the Fig. 2C the further apart from the origo a spot is, the clearer the separation between the groups, and the more suitable the finding would be as a biomarker. All figures indicate differences between the protein allergen (Protein) and isocyanate (Isocyanate) groups and the healthy controls (Control), whereas the welding group (Welding) shows the least up- or down-regulation to healthy persons. Immunoblot analysed gel spots are highlighted in Fig. 2C.

Figure 3 Comparison of protein abundance by Western blotting. 2D-DIGE gel spot intensities for Glutathione S-transferase 1 (GSTP1, mean of several spots/gel), Galectin 10 (LEG10, one gel spot), Protein S100-A9 (S10A9, mean of several spots/gel) and Calcyphosin (CAYP1, one gel spot) of the 32 nasal brush samples (NBS) are on the left. The corresponding protein levels in the Western blot analysis for 82 NBS are on the right. Bars indicate means. Logaritmic transformation was used to attain normal distribution of the variables. The differences between the groups were analysed using the Anova and Fisher's LSD test for post hoc comparisons. Control = healthy persons, Protein= protein allergens group; Isocyanate= isocyanate group; Welding= welding group.

Figure 1.

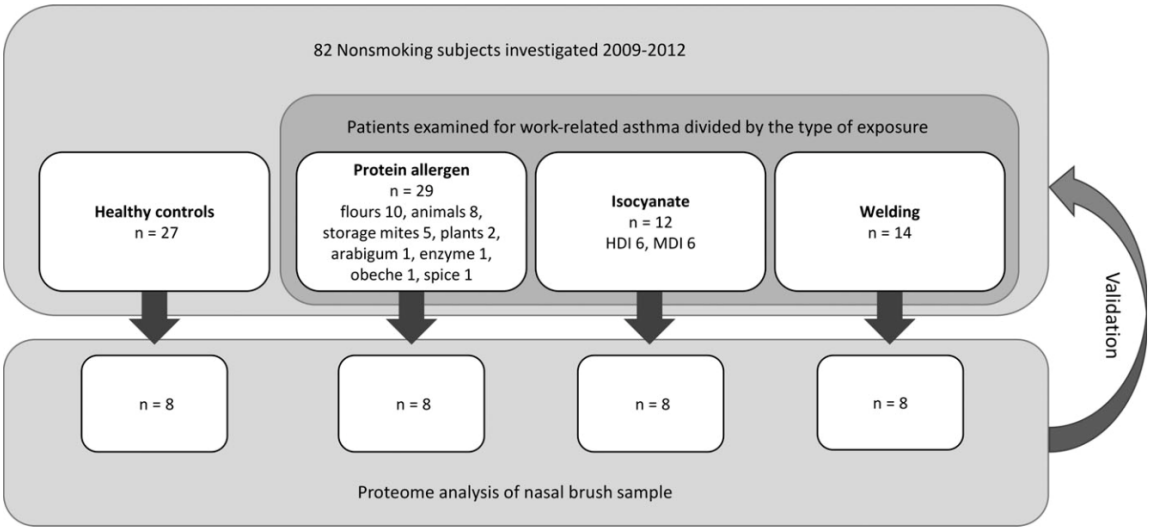
82 Nonsmoking subjects investigated 2009-2012
Patients examined for work-related asthma divided by the type of exposure
Healthy controls
n = 27
Protein allergen
n = 29
flours 10, animals 8,
storage mites 5, plants 2,
arabigum 1, enzyme 1,
obeche 1, spice 1
Isocyanate
n = 12
HDI 6, MDI 6
Welding
n = 14
Validation
n = 8
n = 8
n = 8
n = 8
Proteome analysis of nasal brush sample

Figure 2.

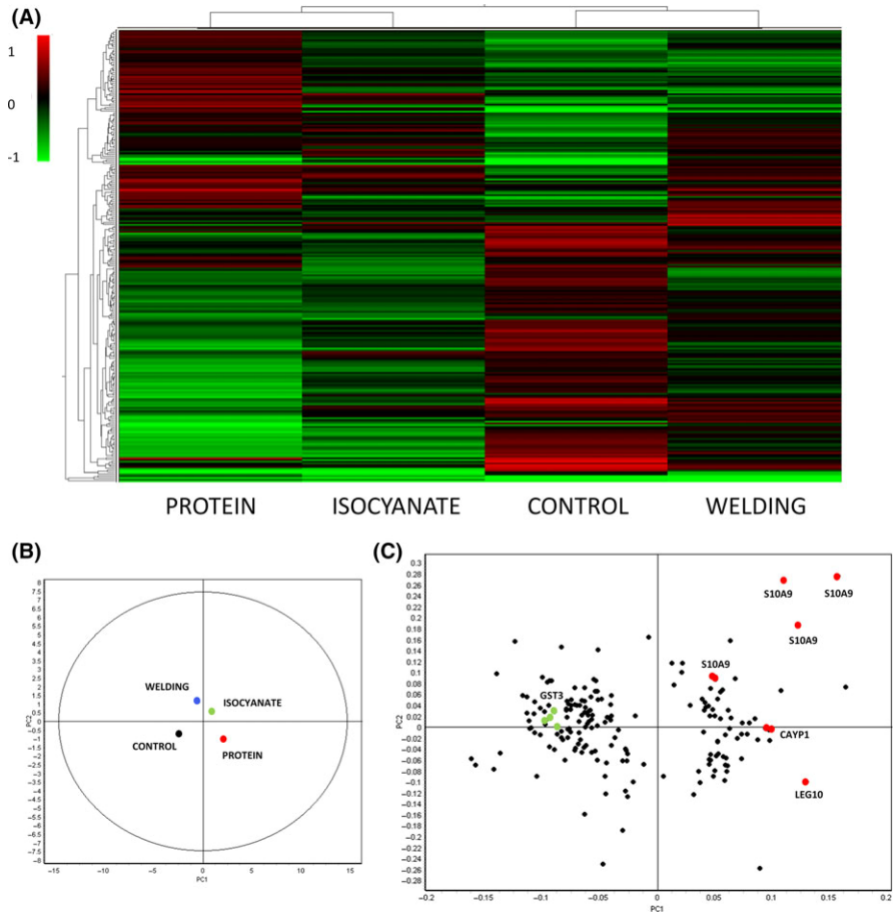
(A)
1
0
-1
PROTEIN
ISOCYANATE
CONTROL
WELDING
(B)
WELDING
ISOCYANATE
CONTROL
PROTEIN
PC1
PC2
(C)
S10A9
S10A9
S10A9
S10A9
GST3
CAYP1
LEG10
PC1
PC2

Figure 3.

Supplementary data

Supplementary material 1
Methods

Skin prick tests and IgE measurements
The skin prick test (SPT) panel for common environmental allergens included a negative control, a positive control (histamine), and 11 standardized environmental antigens (ALK-Abello, Hørsholm, Denmark). Occupational protein allergens were tested with commercial extracts, as is, or as an in-house extract in potassium phosphate, depending on the agent. Isocyanates were conjugated with human serum albumin (1), and metals ($CoCl_2$, $NiSO_4$, K_2CrO_4, $CrCl_2$) were diluted in aqua. The results were regarded as positive if the mean wheal diameter was ≥ 3 mm, and the negative control wheal was < 2 mm. Serum total and specific (protein allergens and isocyanates) IgE were measured using the Phadia UniCAP System (Phadia, Uppsala, Sweden). A specific IgE of < 0.35 kU/l was considered normal.

Lung function, exhaled and nasal nitric oxide
Flow-volume spirometry was performed using a standard spirometer (Spirostar USB Medikro, Finland), and the predicted values of the Finnish population. Nonspecific airway hyperresponsiveness was measured using the histamine challenge (2). Exhaled (FeNO) and nasal (nNO) nitric oxide were measured using an online chemiluminescence analyser (NIOX, Aerocrine AB, Solna, Sweden) in compliance with recommendations (3).

Controlled exposure to occupational agent
The WRA patients were exposed to the relevant occupational agent for 15–30 minutes approximately 24 h before sample collection, by mimicking work tasks in a 6 m^3 challenge chamber or by nebulizing allergen extracts (animal dander, storage mites), in-house solutions (Methylene diphenyl diisocyanate) or occupational agents (Hexamethylene diisocyanate) (4).

Nasal brush and induced sputum samples
The NBS was taken from the nasal cavity's middle meatus. The brush was dipped into 5 mL of cold PBS, mixed gently and centrifuged at 500g. Supernatant was filtered through a 0.45 µm membrane, divided into aliquots and frozen to -70ºC for further use. Sputum was induced with hypertonic saline, in accordance with the guidelines (5). The number of eosinophils /200 cells were counted in the sputum and the NBS smear samples.

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3 Ats/Ers. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005: **171**: 912-30.

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5 Djukanovic R, Sterk P J, Fahy J V, Hargreave F E. Standardised methodology of sputum induction and processing. *Eur Respir J Suppl* 2002: **37**: 1s-2s.

Table S1. Characteristics of 32 participants whose nasal brush samples were included in proteomic analysis. Continuous variables were expressed as means (± standard deviation) or median (interquartile range) depending on their distribution and categorical values as percentages. Logaritmic transformation was used to attain normal distribution of continuous variables. The differences between the groups were analysed using the ANOVA, Kruskal-Wallis test (when normal distribution was not attained after logarithmic transformation) or Chi-square test.

	Healthy controls (n=8)	**Asthma related to protein allergen (n=8)**	**isocyanate (n=8)**	**welding (n=8)**	**p**
Age	43.8 (2.1)	35.6 (12.3)	41.7 (11.4)	43.3 (10.4)	0.349
Sex, male, n (%)	8 (100.0)	1 (12.5)	6 (75.0)	6 (75.0)	0.002
Duration of work exposure, years, median (Q_1-Q_3) [#]	NA	7.5 (5.3-25.5)	6.5 (2.3-13.8)	21.5 (6.3-25.8)	0.265
Duration of asthma symptoms, years, median (Q_1-Q_3) [¤]	NA	3.0 (2.3-4.0)	1.0 (0.7-2.8)	3.5 (1.3-9.8)	0.032
VAS rhinitis, mm, median (Q_1-Q_3) [#]	10.5 (0.0-16.3)	57.5 (11.8-66.8)	20.0 (0.0-50.0)	7.5 (0.0-38.5)	0.083
VAS nasal congestion, mm, median (Q_1-Q_3) [#]	5.0 (0.0-20.5)	49.5 (30.3-71.3)	80.0 (30.0-84.0)	14.5 (2.3-45.5)	0.021
VAS nasal itching, mm, median (Q_1-Q_3) [#]	0.0 (0.0-0.0)	45.0 (16.8-59.8)	60.0 (0.0-80.0)	4.5 (0.3-16.3)	0.002
Nasal steroid during 4 weeks, n (%)	0 (0.0)	1 (12.5)	1 (12.5)	2 (25.0)	0.515
Inhaled steroid during 4 weeks, n (%)	0 (0.0)	3 (37.5)	5 (62.5)	8 (100.0)	0.001
≥1 positive SPT to common environmental allergen, n (%)	0 (0.0)	7 (87.5)	3 (37.5)	4 (50.0)	0.005
Positive SPT to occupational allergen, n (%)	NA	8 (100.0)	0 (0.0)	0 (0.0)	<0.001
Total IgE, kU/l, median (Q_1-Q_3) [¤]	16.5 (8.8-23.3)	143.0 (42.8-301.8)	40.0 (17.5-63.5)	95.5 (32.8-203.0)	0.007
Blood eosinophils 10^6/l, mean (SD)	128.8 (86.8)	255.0 (166.2)	183.8 (172.5)	207.5 (129.9)	0.374
FVC % predicted, mean (SD)	98.6 (8.1)	99.9 (10.1)	97.3 (14.1)	94.9 (10.7)	0.821
FEV1 % predicted, mean (SD)	97.9 (9.8)	92.6 (9.5)	92.5 (8.5)	91.0 (12.9)	0.573
FeNO, ppb, median (Q_1-Q_3) [¤]	12.8 (11.1-16.9)	32.3 (18.5-69.4)	15.8 (10.4-25.1)	18.8 (10.5-39.9)	0.009
nNO, ppb, mean (SD)	858.7 (263.4)	1006.7 (358.1)	694.4 (233.6)	927.1 (370.0)	0.249
Sputum eosinophil %, median (Q_1-Q_3), n=20 [#]	0.0 (0.0-0.3)	3.0 (0.5-6.5)	1.0 (0.0-2.0)	0.5 (0.0-1.0)	0.080
Nasal eosinophil %, median (Q_1-Q_3) [#]	0.0 (0.0-0.0)	0.5 (0.0-1.75)	1.0 (0.0-4.0)	0.0 (0.0-0.0)	0.056

[#] Kruskal-Wallis test was used; [¤]Logaritmic transformation was used; *VAS*, Visual analogue scale of nasal symptoms within a week; *SPT*, skin prick test; *IgE*, Immunoglobulin E; *FVC*, forced vital capacity; FEV_1, Forced expiratory volume in one second; FeNO, exhaled nitric oxide; nNO, nasal nitric oxide.

Table S2

Table S2. List of identified proteins from 2D-DIGE analysis of nasal brush samples. Proteins were identified from the picked 228 gel spots by tandem mass spectrometry with subsequent database searches using Proteome Discover software. Student's t-test and the Average (Av.) Ratio (with significance levels of < 0.05 and Av. Ratio $\geq |1.5|$, respectively) for comparing inhalation challenged protein, isocyanate and welding groups and healthy controls to one another were obtained from DeCyder software.

											Protein vs Control		Isocyanate vs Control		Welding vs Control		Protein+Isocyanate+ Welding vs Control		Welding vs Protein		Isocyanate vs Welding		Isocyanate vs Protein	
Gel spot #	UniProt Accession	Identification	UniProt Entry Name	Mascot score	Sequest score [a]	Sequence Coverage % [b]	Peptides [c]	MW (kDa) [d]	pI [e]		T-test	Av.Ratio	T-test	Av.Ratio	T-test	Av. Ratio	T-test	Av. Ratio	T-test	Av. Ratio	T-test	Av. Ratio	T-test	Av. Ratio
3	P01023	Alpha-2-macroglobulin	A2MG		27,61	6,72	9	163,1879	6,46										0,0079	-1,85				
9	P01023	Alpha-2-macroglobulin	A2MG	134,75		1,29	2	163,1879	6,46		0,015	1,83							0,0043	-1,93				
53	P08603	Complement factor H	CFAH		0	9,10	9	138,9787	6,62								0,023	1,62						
66	P01024	Complement C3; alpha-chain	CO3		17,53	3,37	6	111,5	6,40										0,041	-1,75				
103	P01024	Complement C3; alpha-chain	CO3	122,61		1,62	3	111,5	6,40		0,036	1,82							0,031	-1,71				
108	P01023	Alpha-2-macroglobulin	A2MG		91,67	25,85	28	163,1879	6,46		0,005	2,11							0,038	-1,7			0,02	-1,83
	P01024	Complement C3; alpha-chain	CO3		54,64	20,32	27	111,5	6,40															
159	P00751	Complement factor B	CFAB		1,82	7,20	4	85,47853	7,06		0,027	1,88	0,033	1,88			0,01	1,75						
185	P01833	Polymeric immunoglobulin receptor	PIGR	538,18		19,37	11	83,23166	5,74				0,024	1,55							0,027	1,58		
	P22314	Ubiquitin-like modifier-activating enzyme 1	UBA1	292,64		8,98	7	117,7743	5,76															
282	P01833	Polymeric immunoglobulin receptor	PIGR	595,07		15,71	11	83,23166	5,74				0,042	-2,43	0,033	-2,47			0,018	-2,01			0,0034	-1,98
	O43707	Alpha-actinin-4	ACTN4	557,60		12,84	11	104,7885	5,44															
353	P01023	Alpha-2-macroglobulin	A2MG	279,60		6,51	8	163,1879	6,46														0,0053	-2,19
354	P02787	Serotransferrin	TRFE	589,26		35,67	20	77.01	7,12		0,048	3,1	0,035	1,84	0,0087	1,63	0,02	2,18						
	Q08188	Protein-glutamine gamma-glutamyltransferase	TGM3	521,24		20,78	13	76,58359	5,86															
382	P15311	Ezrin	EZRI	588,67		17,24	13	69,36974	6,27												0,029	-1,94	0,023	-1,96
399	O43707	Alpha-actinin-4	ACTN4	757,34		14,05	10	104,7885	5,44				0,026	-1,77	0,024	-1,95	0,026	-1,59						
400	P02768	Serum albumin	ALBU	503,77		15,44	8	69,3215	6,28		0,0035	3,17	0,0071	2,81	0,022	2,36	0,00016	2,8						
464	P15311	Ezrin	EZRI	1165,71		20,82	13	69,36974	6,27														0,0017	-1,67
484	P01833	Polymeric immunoglobulin receptor	PIGR	89,92		3,40	3	83,23166	5,74		0,0077	1,85							0,0078	-2,18				
513	P01833	Polymeric immunoglobulin receptor	PIGR	1085,85		24,35	15	83,23166	5,74										0,02	-2,11				
514	P02768	Serum albumin	ALBU	256,71		4,60	3	69,3215	6,28										0,027	-2,07				
537	P02788	Lactotransferrin	TRFL	65,43	8,02	2,25	2	78,13192	8,12		0,018	2,53							0,047	-1,94				
543	P02788	Lactotransferrin	TRFL	2620,00		47,89	31	78,13192	8,12		0,009	2,22					0,023	2,01						
547	P02768	Serum albumin	ALBU	972,85		14,94	11	69,3215	6,28		0,018	1,82											0,02	-1,64
	P01833	Polymeric immunoglobulin receptor	PIGR	227,52		5,24	4	83,23166	5,74															
552	Q08188	Protein-glutamine gamma-glutamyltransferase	TGM3	521,24		20,78	13	76,58359	5,86		0,042	2												
553	P02788	Lactotransferrin	TRFL	2916,33		44,37	29	78,13192	8,12		0,018	2,52					0,01	1,95						
588	P02768	Serum albumin	ALBU	198,65		7,22	5	69,3215	6,28										0,0015	-1,7				
592	P02768	Serum albumin	ALBU	3758,28		46,14	30	69.32	6,28						0,044	1,54	0,022	1,57						
602	P02787	Serotransferrin	TRFE	983,48		34,10	23	77,01363	7,12		0,0088	2,3					0,038	1,67	0,016	-2				
	P01871	Ig mu chain C region	IGHM	626,10		23,45	9	(49.3)	6,77															
	P16050	Arachidonate 15-lipoxygenase	LOX15	316,44		13,90	9	74,75652	6,58															
603	P02788	Lactotransferrin	TRFL	257,69		6,48	4	78,13192	8,12		0,023	2,06					0,011	1,73	0,035	-1,7				
608	P02788	Lactotransferrin	TRFL	9208,64		70,14	52	78,13192	8,12		0,00096	5,43	0,012	4,95	0,018	2,87	0,00013	4,51	0,0035	-1,89				
624	P02787	Serotransferrin	TRFE	3446,42		40,54	31	77,01363	7,12		0,022	2,01							0,022	-1,93				
	P01871	Ig mu chain C region	IGHM	1057,51		26,33	10	(49.3)	6,77															
	P16050	Arachidonate 15-lipoxygenase	LOX15	513,01		22,66	15	74,75652	6,58															
629	P02788	Lactotransferrin	TRFL	3986,27		34,96	23	78,13192	8,12		0,011	2,04							0,016	-1,97				
630	P01871	Ig mu chain C region	IGHM	176,98		5,97	3	(49.3)	6,77		0,014	1,89							0,04	-1,68				
633	P02787	Serotransferrin	TRFE	737,05		31,23	21	77,01363	7,12		0,013	1,73							0,012	-1,76				
	P01871	Ig mu chain C region	IGHM	230,18		9,29	4	(49.3)	6,77															
637	P02787	Serotransferrin	TRFE	15912,88		69,63	64	77,01363	7,12		0,034	1,75							0,032	-1,71				
638	P02768	Serum albumin	ALBU	2671,67		30,38	19	69,3215	6,28		0,045	1,58							0,002	-1,78			0,0076	-1,6
660	P02768	Serum albumin	ALBU	1327,98		18,56	13	69,3215	6,28										0,029	-1,64				
	P11142	Heat shock cognate 71 kDa protein	HSP7C	684,85		18,58	11	70,85423	5,52															
665	P11142	Heat shock cognate 71 kDa protein	HSP7C	4584,98		34,83	24	70,85423	5,52						0,025	-1,84								
668	P02768	Serum albumin	ALBU	2832,91		23,15	16	69,3215	6,28										0,024	-1,67				
	P02790	Hemopexin	HEMO	117,65		4,76	2	51,64328	7,02															
689	P11142	Heat shock cognate 71 kDa protein	HSP7C	17471,84		45,51	33	70,85423	5,52						0,025	-1,84	0,007	-1,94						
692	P11142	Heat shock cognate 71 kDa protein	HSP7C	5313,46		39,47	27	70,85423	5,52						0,0058	-2,49								
728	P11142	Heat shock cognate 71 kDa protein	HSP7C	2054,24		33,75	24	70,85423	5,52						0,024	-2,15	0,02	-1,82						
	P02768	Serum albumin	ALBU	2018,43		39,74	26	69,3215	6,28															
735	P01024	Complement C3; beta chain	CO3	5760,72		21,47	38	(187,03)	6,40		0,02	1,69					0,012	1,6						
738	P01024	Complement C3;beta chain	CO3	3595,60		23,99	33	(187,03)	6,40										0,016	-1,89				
790	P07237	Protein disulfide-isomerase	PDIA1	620,17		13,58	6	57,08068	4,87												0,0052	2,07		
801	P07237	Protein disulfide-isomerase	PDIA1	2699,54		25,79	14	57,08068	4,87				0,0073	1,67										
810	P36871	Phosphoglucomutase-1	PGM1	320,71		17,97	9	61,41053	6,76		0,045	1,67												
	P00751	Complement factor B	CFAB	296,29		5,37	4	85,47853	7,06															
813	P42357	Histidine ammonia-lyase	HUTH	365,92		19,94	10	72,65183	6,95				0,029	1,74										
842	P14618	Pyruvate kinase isozymes M1/M2	KPYM	3398,92		40,30	21	57,90003	7,84										0,0029	1,9	0,023	-1,55		
851	P06744	Glucose-6-phosphate isomerase	G6PI	970,34		28,85	13	63,10725	8,32						0,024	2,09	0,027	1,57						
852	P14618	Pyruvate kinase isozymes M1/M2	KPYM	94,91		7,16	3	57,90003	7,84						0,011	2,45					0,023	-2,02		
865	P01857	Ig gamma-1 chain C	IGHG1	1098,75		26,06	8	(36,08)	8,19														0,025	-1,54
882	P01876	Ig alpha-1 chain C region	IGHA1	646,88		14,45	4	(37,63)	6,51		0,0035	2,04												
916	P01009	Alpha-1-antitrypsin	A1AT	1668,40		28,47	11	46,70702	5,59		0,042	2,15							0,035	-2,12				
929	P23381	Tryptophan--tRNA ligase, cytoplasmic	SYWC	1060,52		39,28	17	53,13161	6,23		0,013	-2,18					0,011	-1,8						
	P30838	Aldehyde dehydrogenase	AL3A1	856,43		28,70	11	50,36285	6,54															
	Q13228	Selenium-binding protein 1	SBP1	708,88		41,95	18	52,35763	6,37															
932	P05091	Aldehyde dehydrogenase, mitochondrial	ALDH2	583,59		19,15	11	56,34563	7,05						0,012	-1,68								
	Q13228	Selenium-binding protein 1	SBP1	423,81		21,19	10	52,35763	6,37															
936	P12081	Histidine--tRNA ligase, cytoplasmic	SYHC	867,12		34,38	16	57,37422	5,88		0,038	-1,57							0,017	1,55				
941	P23381	Tryptophan--tRNA ligase, cytoplasmic	SYWC	1122,89		32,70	14	53,13161	6,23		0,008	-1,67												
942	Q13228	Selenium-binding protein 1	SBP1	3077,83		35,38	17	52,35763	6,37		0,011	-1,93												
943	P30838	Aldehyde dehydrogenase	AL3A1	3003,11		36,42	14	50,36285	6,54		0,0033	-2,76	6,40E-05	-3,4	0,015	-2,19	0,00062	-2,7						
946	Q13228	Selenium-binding protein 1	SBP1	356,58		12,92	5	52,35763	6,37		0,00023	-2,05	0,013	-1,58			0,011	-1,51	0,012	1,84				
949	P68371	Tubulin beta-4B	TBB4B	1019,53		24,27	10	49,799	4,89														0,038	-1,69
951	P30838	Aldehyde dehydrogenase	AL3A1	6555,53		43,05	18	50,36285	6,54		0,023	-2,21	0,036	-2,33			0,022	-1,92						
	Q13228	Selenium-binding protein 1	SBP1	3491,67		65,25	29	52,35763	6,37															
	P00352	Retinal dehydrogenase 1	AL1A1	2463,28		47,90	21	54,82695	6,73															
953	P48637	Glutathione synthetase	GSHB	312,03		8,02	5	52,35226	5,92		0,0063	-2,08					0,043	-1,53						
955	P30838	Aldehyde dehydrogenase	AL3A1	340,27		10,38	5	50,36285	6,54		0,0013	-2,74	0,014	-2,28			0,0041	-2,04	0,038	1,87				
956	P02768	Serum albumin	ALBU	2744,40		29,23	22	69,3215	6,28		0,0006	-4,75					0,013	-2,17	0,0051	3,62			0,015	2,12
	P30838	Aldehyde dehydrogenase	AL3A1	1287,45		41,72	19	50,36285	6,54															
959	P68104	Elongation factor 1-alpha 1	EF1A1	550,07		12,12	2	50,10911	9,01		0,03	-1,94					0,048	-1,51						
	P04406	Glyceraldehyde-3-phosphate dehydrogenase	G3P	402,13		18,51	2	36,0304	8,46															
960	P04406	Glyceraldehyde-3-phosphate dehydrogenase	G3P	1037,57		18,51	2	36,0304	8,46		0,026	-1,43	0,03	-1,5										
	P68104	Elongation factor 1-alpha 1	EF1A1	918,53		17,10	4	50,10911	9,01															
964	P00352	Retinal dehydrogenase 1	AL1A1	5359,22		48,10	28	54,82695	6,73		0,022	-2,25							0,0087	1,91	0,042	-1,57		
975	P00352	Retinal dehydrogenase 1	AL1A1	1013,50		11,18	6	54,82695	6,73		0,043	-2,27							0,03	1,89				
987	P48637	Glutathione synthetase	GSHB	2496,22		41,77	16	52,35226	5,92		0,032	-1,69							0,00072	1,98	0,02	-1,67		
993	P02679	Fibrinogen gamma chain	FIBG	1971,19		40,40	15	51,47887	5,62										0,017	1,53				
998	Q13867	Bleomycin hydrolase	BLMH	672,78		30,11	15	52,52802	6,27		0,037	-1,86					0,0096	-1,79						
	P50395	Rab GDP dissociation inhibitor beta	GDIB	635,21		58,43	21	50,63088	6,47															
	P04040	Catalase	CATA	591,11		32,64	16	59,71876	7,39															
1004	P50395	Rab GDP dissociation inhibitor beta	GDIB	1563,65		68,54	28	50,63088	6,47		0,0013	-2,51	0,018	-2	0,019	-1,71	0,0023	-2,02						
1008	P02679	Fibrinogen gamma chain	FIBG	1742,41		18,32	9	51,47887	5,62										0,003	1,71	0,032	-1,56		

1019	P00738	Haptoglobin	HPT	461,47		26,11	9	(45,18)	6,58											0,007
1024	P04406	Glyceraldehyde-3-phosphate dehydrogenase	G3P	588,61		46,87	11	36,0304	8,46	0,034	-1,51	0,0088	-1,55							
1026	P50395	Rab GDP dissociation inhibitor beta	GDIB	133,39		15,06	6	50,63088	6,47	0,01	-1,89	0,015	-1,75			0,011	-1,6			
1028	P52209	6-phosphogluconate dehydrogenase	6PGD	220,86		12,84	5	53,10596	7,23			0,03	2,32	0,024	2	0,0074	2,11			
1031	P26641	Elongation factor 1-gamma	EF1G	343,48		18,76	7	50,08714	6,67	0,013	-2,11							0,022	1,67	
	P06733	Alpha-enolase	ENOA	287,04		14,75	5	47,13932	7,39											
1033	P06733	Alpha-enolase	ENOA	1559,68		48,39	16	47,13932	7,39											0,031
1037	P04040	Catalase	CATA	257,31		12,90	6	59,71876	7,39	0,022	1,77	0,046	2,87			0,005	2,35			
1039	P25311	Zinc-alpha-2-glycoprotein	ZA2G	688,74		33,56	11	34,23711	6,05	0,067	1,56	0,013	1,63							
1040	P29508	Serpin B3	SPB3	113,08		7,44	3	44,53655	6,81	0,0021	-2,02	0,039	-1,92	0,017	-1,72	0,0032	-1,88			
1062	P23526	Adenosylhomocysteinase	SAHH	274,84		23,15	10	47,68521	6,34	0,004	-1,91	0,032	-1,7	0,017	-1,76	0,0019	-1,79			
	P50395	Rab GDP dissociation inhibitor beta	GDIB	197,84		26,52	10	50,63088	6,47											
1065	P23526	Adenosylhomocysteinase	SAHH	1382,91		24,54	10	47,68521	6,34									0,033	1,56	0,008
1081	O00231	26S proteasome non-ATPase regulatory subunit 11	PSD11	1098,05		51,90	20	47,43404	6,48	0,0081	-1,58									
1083	P25311	Zinc-alpha-2-glycoprotein	ZA2G	787,74		24,83	7	34,23711	6,05	0,0038	2,27					0,015	1,77	0,045	-1,59	
	P00738	Haptoglobin	HPT	371,21		12,81	6	(45,18)	6,58											
1087	P25311	Zinc-alpha-2-glycoprotein	ZA2G	795,44		42,62	15	34,23711	6,05							0,023	1,8			
	P00738	Haptoglobin	HPT	315,12		25,12	10	(45,18)	6,58											
1104	P00738	Haptoglobin	HPT	1314,18		29,80	11	(45,18)	6,58	0,022	2,02									
	P25311	Zinc-alpha-2-glycoprotein	ZA2G	994,76		54,03	15	34,23711	6,05											
1116	O75874	Isocitrate dehydrogenase	IDHC	643,12		26,33	11	46,62952	7,01	0,014	-2,74							0,00065	2,6	
1118	P00738	Haptoglobin	HPT	347,65		17,49	9	(45,18)	6,58	0,007	1,83							0,041	-1,53	
	P25311	Zinc-alpha-2-glycoprotein	ZA2G	296,59		15,10	4	34,23711	6,05											
1120	P00966	Argininosuccinate synthase	ASSY	100,16		5,58	3	46,50097	8,02	0,013	-2,79							0,036	3,19	
	P29508	Serpin B3	SPB3	87,53		4,87	2	44,53655	6,81											
1126	P12277	Creatine kinase B-type	KCRB	3000,36		65,35	18	42,61732	5,59	0,02	1,71							0,0021	-2,09	
1173	P04075	Fructose-bisphosphate aldolase A	ALDOA	560,17		24,45	9	39,39531	8,09	0,00014	-2,53					0,0087	-1,73	0,047	1,81	
1188	P29508	Serpin B3	SPB3	1648,09		48,72	20	44,53655	6,81	0,038	-1,94							0,0088	1,79	
1189	P06733	Alpha-enolase	ENOA	270,99		20,74	8	47,13932	7,39	0,031	-1,85							0,024	2,67	
	P29508	Serpin B3	SPB3	105,87		8,46	4	44,53655	6,81											
1196	P30740	Leukocyte elastase inhibitor	ILEU	4670,93		46,97	20	42,7147	6,28	0,023	-1,56									
1199	P29508	Serpin B3	SPB3	363,00		14,00	5	44,53655	6,81	0,018	-3,92							0,0037	4,49	
1217	P01009	Alpha-1-antitrypsin	A1AT	1520,36		13,40	5	46,70702	5,59											
1229	P04075	Fructose-bisphosphate aldolase A	ALDOA	1022,93		43,96	12	39,39531	8,09	0,04	-2,36							0,05	2,09	
1244	P04075	Fructose-bisphosphate aldolase A	ALDOA	1298,78		46,43	13	39,39531	8,09	0,14	-1,76									
	P07355	Annexin A2	ANXA2	389,76		28,91	10	38,57982	7,75											
1251	P04075	Fructose-bisphosphate aldolase A	ALDOA	2210,09		71,98	20	39,39531	8,09	0,0027	-2,57					0,011	-1,92			
1253	Q9NR45	Sialic acid synthase	SIAS	1127,70		39,00	8	40,28146	6,74									0,022	2	
1259	P07355	Annexin A2	ANXA2	219,41		31,56	9	38,57982	7,75	0,037	-1,77							0,035	2,12	
	P04406	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	211,44		31,64	9	36	8,50											
1264	Q13630	GDP-L-fucose synthase	FCL	228,42		26,79	7	35,86996	6,60	0,091	-2,76							0,025	4,05	
	P29508	Serpin B3	SPB3	197,84		21,03	7	44,53655	6,81											
1268	P29508	Serpin B3	SPB3	5631,00		54,10	28	44,53655	6,81									0,049	3,33	
1269	P14324	Farnesyl pyrophosphate synthase	FPPS	193,36		12,41	4	48,24463	6,15							0,048	-1,5			
1274	P04083	Annexin A1	ANXA1	151,43		16,76	4	38,68998	7,02			0,03	-2,4							
1288	P04075	Fructose-bisphosphate aldolase A	ALDOA	267,83		23,90	6	39,39531	8,09	0,011	-2,3							0,041	1,82	
1297	Q13630	GDP-L-fucose synthase	FCL	1109,54		34,58	10	35,86996	6,60	0,048	-1,97									
1316	P05120	Plasminogen activator inhibitor 2	PAI2	608,47		31,81	11	46,56615	5,63	0,032	-1,8									
1318	P37837	Transaldolase	TALDO	675,14		37,39	12	37,51646	6,81	0,0022	-2,25									
	P04083	Annexin A1	ANXA1	651,81		41,04	12	38,68998	7,02											
1320	P14550	Alcohol dehydrogenase [NADP(+)]	AK1A1	4263,42		59,38	20	36,54986	6,79	0,023	-3,06									
1334	P37837	Transaldolase	TALDO	1451,16		40,95	13	37,51646	6,81	0,008	-2,43									
	P04083	Annexin A1	ANXA1	701,44		46,24	13	38,68998	7,02											
1344	P07355	Annexin A2	ANXA2	1471,42		39,23	14	38,57982	7,75	0,011	-2,81							0,024	2,93	
1346	P04083	Annexin A1	ANXA1	86,32	1,89	8,09	3	38,68998	7,02							0,043	-2,1			
1347	P04083	Annexin A1	ANXA1	740,72		50,87	15	38,68998	7,02	0,032	-3,16									
	P07355	Annexin A2	ANXA2	951,28		37,17	12	38,57982	7,75											
1351	P04083	Annexin A1	ANXA1	748,59		36,13	14	38,68998	7,02	0,0069	-2,89					0,024	-2,05			
1364	P07355	Annexin A2	ANXA2	3958,43		50,44	20	38,57982	7,75	0,01	-3,3	0,045	-2,27			0,013	-2,39			
1374	P04083	Annexin A1	ANXA1	2987,28	6,94	57,51	18	38,68998	7,02	0,014	-3,02	0,029	-2,72			0,022	-2,2			
1380	P04083	Annexin A1	ANXA1	562,96		24,86	7	38,68998	7,02	0,006	-3,27					0,015	-2,36	0,033	1,91	
1381	P04406	Glyceraldehyde-3-phosphate dehydrogenase	G3P	729,16		49,25	12	36,0304	8,46	0,027	-2,78					0,032	-2,02			
1384	P04083	Annexin A1	ANXA1	1283,34		56,36	18	38,68998	7,02	0,045	-3,78	0,018	-5,05			0,024	-3,22			0,04
	P07355	Annexin A2	ANXA2	628,34		41,89	15	38,57982	7,75											
1386	P04083	Annexin A1	ANXA1	892,71		46,82	13	38,68998	7,02	0,013	-3,15									0,036
1387	P04083	Annexin A1	ANXA1	21054,62		74,28	31	38,68998	7,02	0,022	-2,22									
1388	P04083	Annexin A1	ANXA1	811,37		29,48	12	38,68998	7,02	0,011	-4,59	0,0021	-5,75			0,0049	-3,44			
	P07355	Annexin A2	ANXA2	349,95		27,14	8	38,57982	7,75											
1389	P04406	Glyceraldehyde-3-phosphate dehydrogenase	G3P	1009,00		42,00	15	36	8,50	0,0011	-2,52					0,012	-1,72			
1391	P04083	Annexin A1	ANXA1	1809,38		47,69	17	38,68998	7,02	0,014	-2,36	0,015	-3,99			0,019	-1,87			0,043
1392	P04083	Annexin A1	ANXA1	1106,05		36,71	13	38,68998	7,02	0,025	-3,86	0,032	-2,6			0,012	-2,97			0,05
1393	P04083	Annexin A1	ANXA1	272,92		25,72	8	38,68998	7,02	0,0072	-3,1					0,018	-2,27			
	P07355	Annexin A2	ANXA2	204,37		21,83	6	38,57982	7,75											
1398	P12429	Annexin A3	ANXA3	799,75		41,18	12	36,35266	5,92	0,017	-1,9							0,029	2,1	
1401	P04083	Annexin A1	ANXA1	1633,11		20,52	7	38,68998	7,02							0,039	-2,52			
1433	P04083	Annexin A1	ANXA1	1000,35		42,49	14	38,68998	7,02	0,26	-1,43									
	P00338	L-lactate dehydrogenase A chain	LDHA	492,14		29,22	8	36,66536	8,27											
1434	P00352	Retinal dehydrogenase 1	AL1A1	511,00		9,40	5	54,82695	6,73	0,0047	-1,75									
1437	P0C0L4	Complement C4-A; gamma chain	CO4A	272,16		2,35	4	(192,65)	7,08	0,063	-1,64							0,044	1,58	0,029
1457	P08758	Annexin A5	ANXA5	698,21		39,38	11	35,9144	5,05	0,04	-2,59									
1480	P00352	Retinal dehydrogenase 1	AL1A1	1153,95		11,98	6	54,82695	6,73	0,03	-2,03									
1510	P10768	S-formylglutathione hydrolase	ESTD	245,40		19,50	5	31,44246	7,02	0,0077	-2,18					0,014	-1,84			
1513	P62258	14-3-3 protein epsilon	1433E	710,62		30,98	8	29,15542	4,74	0,0069	-1,91									
	P06753	Tropomyosin alpha-3 chain	TMP3		58,87	35,48	12	29,01475	4,78											
	P67936	Tropomyosin alpha-4 chain	TMP4		31,65	29,84	9	28,50449	4,69											
1523	P09493	Tropomyosin alpha-1 chain	TMP1	355,50		8,45	4	32,68868	4,74	0,033	-1,9					0,038	-1,59			
	P67936	Tropomyosin alpha-4 chain	TMP4	332,25		16,53	5	28,50449	4,69											
1550	P07355	Annexin A2	ANXA2	276,98		26,25	8	38,57982	7,75	0,031	-2,22									
1553	P60709	Actin, cytoplasmic 1	ACTB	1004,36		25,87	7	41,70973	5,48	0,044	-1,68									
1554	P62258	14-3-3 protein epsilon	1433E	484,82		17,65	6	29,15542	4,74	0,0024	-2,63	0,014	-2,27	0,045		0,00059	-2,24			
1555	P62258	14-3-3 protein epsilon	1433E	314,18		26,27	7	29,15542	4,74	0,042	-2,13				-1,9	0,023	-1,87			
1575	O00299	Chloride intracellular channel protein 1	CLIC1	923,99		31,54	9	26,90575	5,17	0,012	-1,8					0,013	-1,71			
1577	O00299	Chloride intracellular channel protein 1	CLIC1	188,83		12,03	3	26,90575	5,17					0,017						
1583	P63104	14-3-3 protein zeta/delta	1433Z	72,41		7,45	2	27,72773	4,79					0,03	-1,56					
1601	P07339	Cathepsin D	CATD	1106,39		25,97	11	44,52363	6,54						-1,67			0,03	1,68	
1619	P63104	14-3-3 protein zeta/delta	1433Z	411,92		35,10	7	27,72773	4,79	0,0088	-1,78									
1620	P31947	14-3-3 protein sigma	1433S	5082,67		38,71	11	27,75669	4,74	0,0071	-1,88									
1660	P63104	14-3-3 protein zeta/delta	1433Z	2551,68		63,27	17	27,72773	4,79	0,0092	-2,21							0,0077	1,8	
	P31946	14-3-3 protein beta/alpha	1433B	1760,23		49,19	5 (14)	28,06483	4,83											
1686	P01834	Ig kappa chain C region	IGKC	619,61		51,89	3	(11.6)	5,87					0,042		0,0094	1,69			
1687	P63104	14-3-3 protein zeta/delta	1433Z	346,62		38,78	8	27,72773	4,79	0,0008	-2,14	0,034	-1,58	0,016	1,62	0,0021	-1,83			
1692	P02743	Serum amyloid P-component	SAMP	170,11		23,77	5	25,37113	6,54	0,043	1,75	0,039	2,01		-1,85	0,012	1,82			
1704	P01834	Ig kappa chain C region	IGKC	242,91		48,11	3	(11.6)	5,87	0,05	1,75					0,038	1,7			
1709	P01834	Ig kappa chain C region	IGKC	281,83		39,45	3	(11.6)	5,87							0,039	1,53			
1711	P28066	Proteasome subunit alpha type-5	PSA5	648,51		52,28	10	26,39421	4,79	0,0063	-1,96					0,036	-1,51			
1715	P02647	Apolipoprotein A-I	APOA1	109,77		16,85	4	30,75893	5,76	0,011	2,4					0,035	1,95			
	P02743	Serum amyloid P-component	SAMP	82,29		14,80	3	25,37113	6,54											
1716	P69905	Hemoglobin subunit alpha	HBA	344,40		25,35	3	15,24793	8,68	0,019	1,78					0,018	1,73			
	P04279	Semenogelin-1	SEMG1	161,74		7,14	3	52,09988	9,29											
1717	A0M8Q6	Ig lambda chain C region	IGLC	325,52		32,08	2	(11.29)	8,28			0,012	2,15			0,0064	1,79			
1743	P04792	Heat shock protein beta-1	HSPB1	935,12		52,20	9	22,76849	6,40	0,029	1,75									
1776	P01834	Ig kappa chain C region	IGKC	243,84		35,85	2	(11.6)	5,87	0,045	1,98	0,011	1,73			0,029	1,79			
1778	A0M8Q6	Ig lambda chain C region	IGLC	506,90		46,23	3	(11.29)	8,28	0,026	1,76									
1828	P01834	Ig kappa chain C region	IGKC	3219,00		80,00	5	(11.6)	5,87	0,027	1,53									
1844	P09211	Glutathione S-transferase P	GSTP1	164,66		10,00	2	23,34102	5,64	0,0079	-1,88					0,04	-1,54	0,017	1,51	
1848	P49862	Kallikrein-7	KLK7	152,98		39,92	6	27,50681	8,47	0,0018	-2,75	0,015	-2,34	0,042	-1,89	0,0024	-2,25			
1855	P09211	Glutathione S-transferase P	GSTP1	1190,55		66,19	11	23,34102	5,64	0,0017	-2,6	0,026	-1,93			0,0077	-1,84			
1856	P09211	Glutathione S-transferase P	GSTP1	748,16		59,05	10	23,34102	5,64	0,0035	-2,55	0,027	-1,99			0,0062	-1,95			
1860	P02747	Complement C1q subcomponent subunit C	C1QC	82,39		15,92	3	25,75714	8,41			0,047	1,6							

1865	P04792	Heat shock protein beta-1	HSPB1	543,80		29,76	5	22,76849	6,40			0,037	1,85							
1868	P09211	Glutathione S-transferase P	GSTP1	1445,67		35,71	5	23,34102	5,64	0,016	-2,85					0,031	-1,99			
1874	Q06830	Peroxiredoxin-1	PRDX1	108,39		25,63	5	22,09628	8,13	0,036	-1,97									
	P13693	Translationally-controlled tumor protein	TCTP	83,17		15,70	2	19,58258	4,93											
1890	Q9NP55	BPI fold-containing family A member 1	BPIA1	150,04		21,09	4	26,69593	5,76			0,034	-1,96							
1892	Q06830	Peroxiredoxin-1	PRDX1	468,34		55,78	10	22,09628	8,13	0,036	-2,21									
1908	P13693	Translationally-controlled tumor protein	TCTP	123,90		24,42	4	19,58258	4,93	0,0022	-1,95	0,011	-1,9	0,045		0,0011	-1,8			
1914	Q06830	Peroxiredoxin-1	PRDX1	1589,03		67,34	15	22,09628	8,13	0,025	-3,46				-1,59					
1920	P32119	Peroxiredoxin-2	PRDX2	560,07		32,32	8	21,87824	5,97	0,0027	-2,5					0,0021	-2			
	P30085	UMP-CMP kinase	KCY	556,65		53,06	8	22,20828	5,57											
1933	Q06830	Peroxiredoxin-1	PRDX1	3490,46		87,94	18	22,09628	8,13									0,019	3,74	
1939	Q06830	Peroxiredoxin-1	PRDX1	98,63		13,07	3	22,09628	8,13	0,024	-3,6					0,04	-2,2	0,016	2,15	
1963	P30086	Phosphatidylethanolamine-binding protein 1	PEBP1	1586,33		50,27	8	21,04367	7,53	0,033	-2,18									
1971	P07741	Adenine phosphoribosyltransferase	APT	364,82		28,33	4	19,59543	6,02	0,048	-3,11									
1976	Q13938	Calcyphosin	CAYP1	364,21		35,45	7	20,95428	4,89			0,031	-2,09			0,045	-1,74			
1978	Q13938	Calcyphosin	CAYP1	1739,67		51,32	13	20,95428	4,89	0,029	-4,76	0,029	-5,15			0,014	-3,72			
1998	P06702	Protein S100-A9	S10A9	1155,94		48,25	6	13,23351	6,13			0,049	-2,98							
	P05109	Protein S100-A8	S10A8	952,00		58,06	8	10,8	6,1											
2013	P12273	Prolactin-inducible protein	PIP	506,38		47,95	6	16,5618	8,05											0,014
2032	P12273	Prolactin-inducible protein	PIP	306,84		66,44	10	16,5618	8,05			0,017	-4,34	0,029						
	P30626	Sorcin	SORCN	276,07		27,27	6	21,66236	5,59											
2036	P00738	Haptoglobin	HPT	177,13		6,16	2	(45.18)	6,58						-2,64			0,014	-2,91	
2037	P00738	Haptoglobin	HPT	334,72		6,90	4	(45.18)	6,58									0,04	-2,03	
2043	P15531	Nucleoside diphosphate kinase A	NDKA	1086,01		63,82	9	17,13767	6,19			0,029	-1,58							
	P00738	Haptoglobin	HPT	285,01		7,39	6	(45.18)	6,58											
2052	P00738	Haptoglobin	HPT	259,32		8,37	4	(45.18)	6,58									0,027	-2,48	
2064	P10599	Thioredoxin	THIO	114,96		12,38	1	11,72974	4,92			0,033	-2,16							
2076	P00441	Superoxide dismutase [Cu-Zn]	SODC	101,62		9,09	1	15,9259	6,13			0,013	-2,63			0,012	-2,06			
2078	P00441	Superoxide dismutase [Cu-Zn]	SODC	1869,73		27,92	4	15,9259	6,13			0,027	-2,32			0,0053	-1,85			
2080	P00441	Superoxide dismutase [Cu-Zn]	SODC	119,94		9,09	1	15,9259	6,13	0,013	-1,98	0,0088	-2,21			0,045	-1,72			
2083	P23528	Cofilin-1	COF1	80,03		6,63	1	18,49066	8,09							0,014	-2,14			
2085	P23528	Cofilin-1	COF1	119,46		18,67	2	18,49066	8,09	0,012	-2,89									
2087	P23528	Cofilin-1	COF1	329,14		35,54	8	18,49066	8,09	E_Hits, E_Isos / Kont						0,023	-2,34			
2095	P37802	Transgelin-2	TAGL2	371,89		24,62	4	22,37717	8,25	0,0038	-3,16	0,042	-2,39			0,035	-1,93	0,00021	2,71	0,019
	P05090	Apolipoprotein D	APOD	405,37		21,16	4	21,26176	5,15											
	P00441	Superoxide dismutase [Cu-Zn]	SODC	213,66		13,64	2	15,9259	6,13											
2098	P23528	Cofilin-1	COF1	121,84		27,11	3	18,49066	8,09	0,0035	-2,13	0,0054	-2,13			0,0064	-1,74			
2108	P00441	Superoxide dismutase [Cu-Zn]	SODC	37,74		23,38	1	15,9259	6,13			0,033	-1,61							
2113	P00441	Superoxide dismutase [Cu-Zn]	SODC	24,61		9,09	1	15,9259	6,13	0,0069	-1,6	0,0025	-1,71							0,039
2128	P00441	Superoxide dismutase [Cu-Zn]	SODC	44,21		9,09	1	15,9259	6,13											0,045
2155	P22392	Nucleoside diphosphate kinase B	NDKB	1035,06		48,68	8	17,28694	8,41	0,025	-2,26							0,046	1,78	
2164	P30044	Peroxiredoxin-5	PRDX5	332,87		37,38	7	22,07255	8,70	0,045	-2,01									
2168	P12273	Prolactin-inducible protein	PIP	1447,58		66,44	9	16,5618	8,05	0,05	2,22					0,023	1,85			
2173	P30044	Peroxiredoxin-5	PRDX5	346,33		36,92	6	22,07255	8,70	0,027	-2,85							0,038	2,07	
2174	P30044	Peroxiredoxin-5	PRDX5	556,23		37,85	7	22,07255	8,70	0,013	-2,07									
	P62937	Peptidyl-prolyl cis-trans isomerase A	PPIA	410,21		60,00	9	18,00089	7,81											
2185	P62937	Peptidyl-prolyl cis-trans isomerase A	PPIA	69,14		17,58	2	18,00089	7,81	0,021	-2,86					0,017	-2,23			
2192	P63241	Eukaryotic translation initiation factor 5A-1	IF5A1	201,06		7,79	1	16,82141	5,24									0,018	3,57	
2216	P63241	Eukaryotic translation initiation factor 5A-1	IF5A1	122,63		26,62	3	16,82141	5,24									0,035	3,7	
2279	P07355	Annexin A2	ANXA2	185,98		20,65	6	38,57982	7,75									0,0088	-2,39	
2282	Q01469	Fatty acid-binding protein 5	FABP5	648,31		37,78	7	15,15455	7,01					0,038	2,69			0,037	2,58	
2298	Q05315	Galectin 10 (Eosinophil lysophospholipase)	LEG10		31,30	4,23	1 (3)	16,44222	7,37	0,029	4,04							0,037	-3,53	
2303	P68871	Hemoglobin subunit beta	HBB	779,66		82,31	10	15,98829	7,28									0,0098	-5,34	
2314	P07737	Profilin-1	PROF1	1155,02		61,43	9	15,04456	8,27									0,016	-1,82	
2318	P06702	Protein S100-A9	S10A9	29,64		11,40	1	13,23351	6,13									0,041	2,13	0,039
	Q01469	Fatty acid-binding protein 5	FABP5	26,53		14,81	2	15,15455	7,01											
2361	P68871	Hemoglobin subunit beta	HBB	296,82		40,14	5	15,98829	7,28									0,044	-5,64	
	P01037	Cystatin-SN	CYTN	233,53		33,33	3	16,37734	7,21											
2362	P68871	Hemoglobin subunit beta	HBB	1402,12		82,99	10	15,98829	7,28					0,043	-2,27			0,021	-6,08	
2371	P06702	Protein S100-A9	S10A9	230,90		26,32	2	13,23351	6,13			0,026	5,9	0,0016	5,26	0,017	4,47			
2387	P06702	Protein S100-A9	S10A9	1537,93		49,12	6	13,23351	6,13			0,047	7,11	0,00023	8,71	0,005	7,01			
2397	P06702	Protein S100-A9	S10A9	296,37		44,74	4	13,23351	6,13					0,017	2,13	0,026	1,89			
2405	P06702	Protein S100-A9	S10A9	1148,47		48,25	7	13,23351	6,13					0,00018	4,92	0,023	1,6			
2411	P06702	Protein S100-A9	S10A9	296,37		105,23	13	13,23351	6,13			0,0084	4,1			0,0016	4,27			
2418	P01040	Cystatin-A	CYTA	238,22		76,53	6	10,99966	5,50	0,015	2,2					0,023	1,71			
	P06702	Protein S100-A9	S10A9	178,22		24,56	2	13,23351	6,13											
2437	P06702	Protein S100-A9	S10A9	6190,53		71,05	14	13,23351	6,13					0,0097	2,14	0,033	1,88			
2463	P06702	Protein S100-A9	S10A9	33,48		11,40	1	13,23351	6,13									0,0098	-3,81	
2509	P05109	Protein S100-A8	S10A8	1716		70	6	10,8	6,1	0,0075	2,43					0,011	1,96	0,015	-1,93	
2528	P02768	Serum albumin	ALBU	7890,68		70,94	46	69,3215	6,28									0,039	-1,8	
	P54652	Heat shock-related 70 kDa protein 2	HSP72	704,29		18,15	11	69,97787	5,74											
2532	P02768	Serum albumin	ALBU	9339,81		59,93	44	69,3215	6,28									0,021	-1,55	
	P02790	Hemopexin	HEMO	1229,12		34,42	16	51,64328	7,02											

a) Raw mass spectrometry data was searched for protein identifications using both Mascot and Sequest programs running in Proteome Discovery. For some of the gel spots Mascot data search did not provide any protein identification, whereas Sequest search prod
b) Sequence Coverage % is how much of the protein sequence was identified by mass spectrometry.
c) Peptides gives the number of high confidence peptides identified by mass spectrometry.
d) Molecular weight (MW) is in parenthesis, when it is not the size of the observed one on the gel. This can be due to cleavage to active chains or proteolysis. For immunoglobins SwissProt database gives only the constant region MW lacking the variable region.
e) pI is the theoretical isoelectric point of the protein.

Table S3

Table S3. IPA enrichment analysis of Top 5 functional categories of the identified 77 proteins				
Category	Function	Function Annotation	p-value	Molecules
Inflammatory Response	inflammation	inflammation of organ	6.90E-21	A2M, ACTB, ALB, ALDOA, ANXA1, ANXA3, ANXA5, APCS, APRT, C3, CAT, CFB, CFL1, CKB, GAPDH, GS
				IGKC, KLK7, LTF, PIP, PPIA, PRDX1, S100A8, SELENBP1, SERPINB1, SERPINB3, SFN, SOD1, TF, TGM3
Cellular Movement	cell movement	cell movement	6.63E-17	A2M, ACTB, ALB, ALDOA, ANXA1, ANXA2, ANXA3, ANXA5, APCS, C3, CAT, CFB, CFL1, GAPDH, HARS, H
				IDH1, IGHM, LTF, NME2, CFL1, GAPDH, HARS, HSPB1, IDH1, IGHM, LTF, NME2, PEBP1, PIGR, PPIA, PR
				S100A8, SELENBP1, SERPINA1, SERPINB1, SERPINB2, SERPINB3, SFN, SOD1, TPT1, WARS, YWHAE,
Free Radical Scavenging	metabolism	metabolism of reactive oxygen species	3.08E-16	ACTB, ALB, ANXA1, APCS, C3, CAT, CFB, CLIC1, GSTP1, HSPB1, IDH1, LTF, PPIA,
				PRDX1, PRDX5, S100A8, SERPINA1, SOD1, TF, YWHAZ
Cell Death and Survival	cell death	cell death	1.39E-15	A2M, ACTB, ALB, ALDH1A1, ALDH3A1, ALDOA, ANXA1, ANXA2, ANXA5, APCS, APRT, C3, CAT, CFB, CFI
				GAPDH, GSS, GSTP1, HSPB1, IGHM, IGKC, LTF, NME2,PEBP1, PIGR, PIP, PPIA, PRDX1, PRDX5,S100A8
				SELENBP1, SERPINA1, SERPINB2, SERPINB3, SFN, SOD1, TF, TGM3, TPT1, TSTA3, YWHAE, YWHAZ
Immunological Disease	allergy	allergy	1.43E-15	ACTB, ALB, ALDOA, ANXA1, ANXA3, ANXA5, C3, CAT, CFL1, GAPDH, IGHM, KLK7, PPIA,
				PRDX1, S100A8, SELENBP1, SERPINB3, TGM3

Table S4. Western blot analysis of nasal brush samples from 50 subjects who were excluded from proteomic analysis. The protein levels of Glutathione S-transferase 1 (GSTP1), Galectin 10 (LEG10), Protein S100-A9 (S10A9) and Calcyphosin (CAYP1) were assessed. Logarithmic transformation was used to attain normal distribution of the variables. The differences between the groups were analysed using the ANOVA and Fisher's LSD test for post hoc comparisons.

	Control, mean, median (Q_1-Q_3) n= 19	Protein allergen, mean, median (Q_1-Q_3) n= 21	Isocyanate, mean, median (Q_1-Q_3) n=4	Welding, mean, median (Q_1-Q_3) n=6	P-value ANOVA	P-value Control vs Protein allergen	P-value Control vs Isocyanate	P-value Control vs Welding	P-value Protein allergen vs Isocyanate	P-value Protein allergen vs Welding	P-value Isocyanate vs Welding
GSTP1	2894147, 1707307 (302617-3922989)	1565380, 970775 (182191-2112343)	4459129, 3503183 (102638-8847821)	3047472, 528945 (132103-4846211)	0.728	NT	NT	NT	NT	NT	NT
LEG10	131978, 48965 (33515-111336)	2343753, 432626 (153697-2025557)	1087008, 511138 (208516-2541371)	2253358, 381704 (38876-4473941)	0.001	<0.001	0.015	0.018	0.859	0.757	0.710
S10A9	518310, 241829 (62877-338919)	1827932, 539315 (127104-2333024)	2691988, 1254421 (480197-6341347)	2696139, 445668 (202302-4560062)	0.037	0.031	0.020	0.065	0.259	0.693	0.500
CAYP1	13202934, 4643285 (1230331-26281878)	3924143, 2810687 (1211475-6702787)	14175095, 6080241 (1084716-35360327)	12081320, 1445428 (383333-18622323)	0.207	NT	NT	NT	NT	NT	NT

Control = healthy persons; Protein= protein allergen group; Isocyanate= isocyanate group; Welding= welding group; Q_1-Q_3=interquartile range; NT= not tested

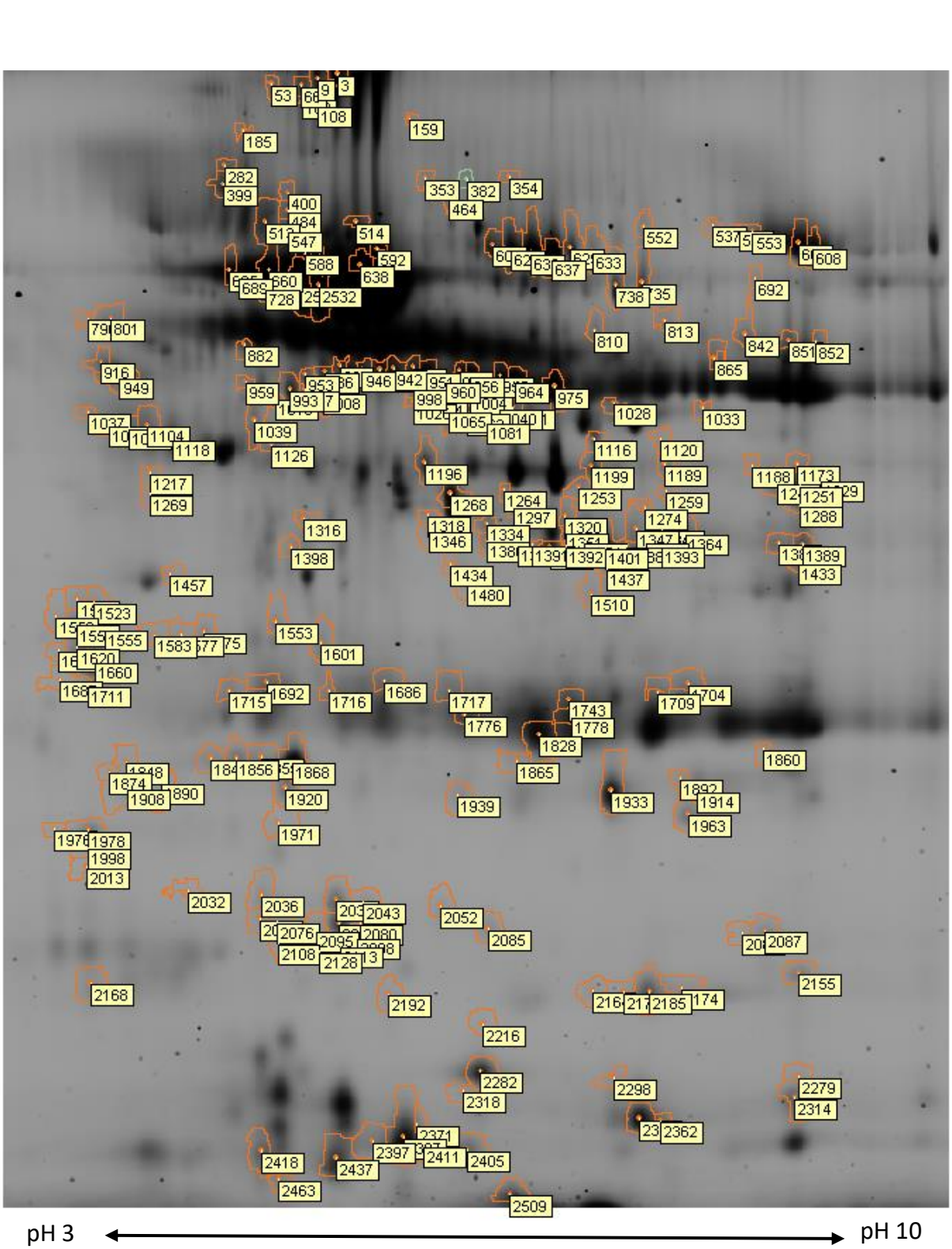


Figure S1 False colour two-dimensional differential gel electrophoresis (2D-DIGE) image of the identifi protein spots in the nasal brush samples. Spot numbers are marked on the gel image and protein identifications are listed in Supplementary file Table S2 according to the spot number.

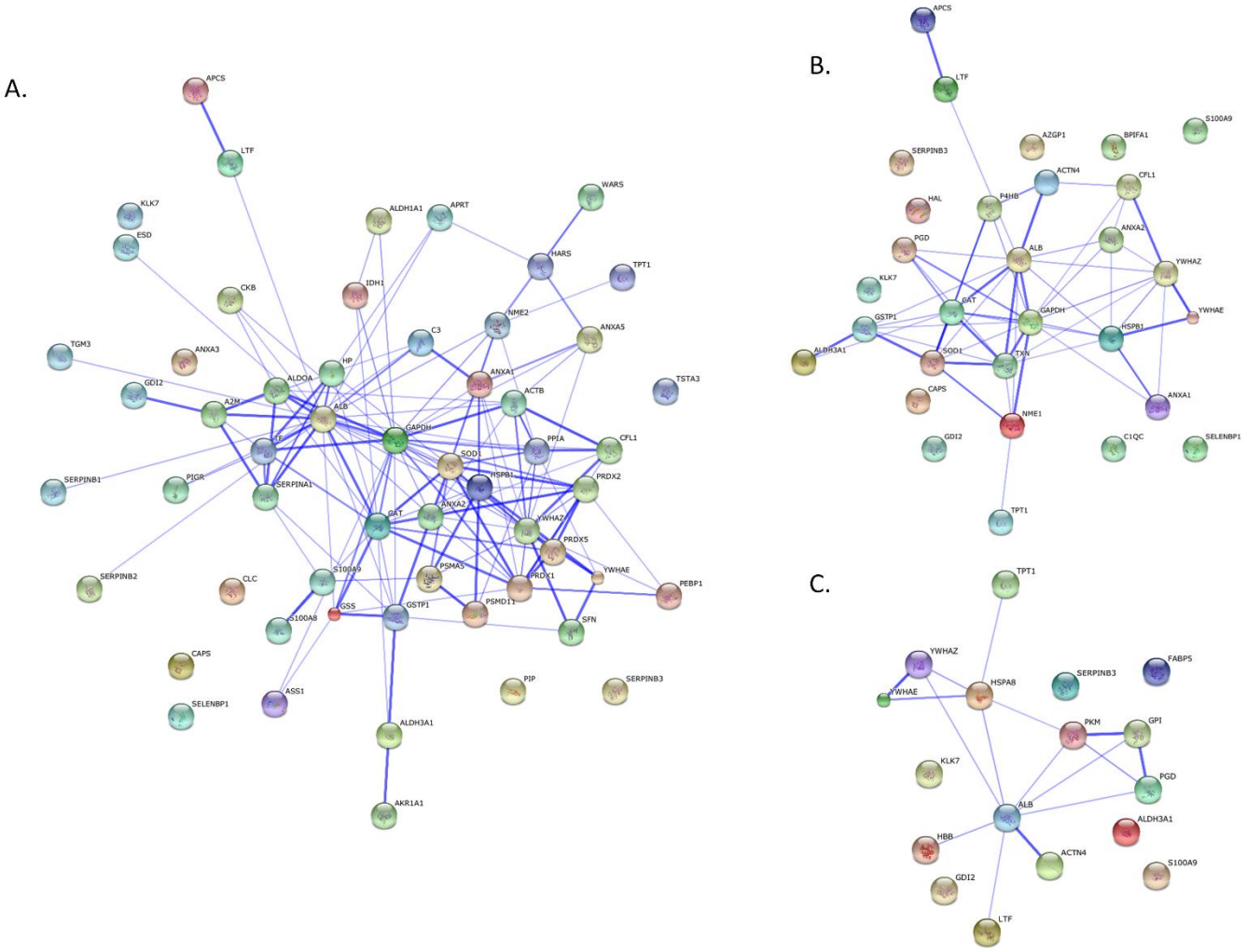


Figure S2 Protein association networks of the work-related asthma groups. Blue lines are confidence-based associations from the Stri database for the identified proteins (Table 2) in the protein allergen (A), isocyanate (B) and welding (C) groups. Stronger associations presented as thicker lines. Minimum required interaction score was 0,4 and all available STRING data sources were applied in the se

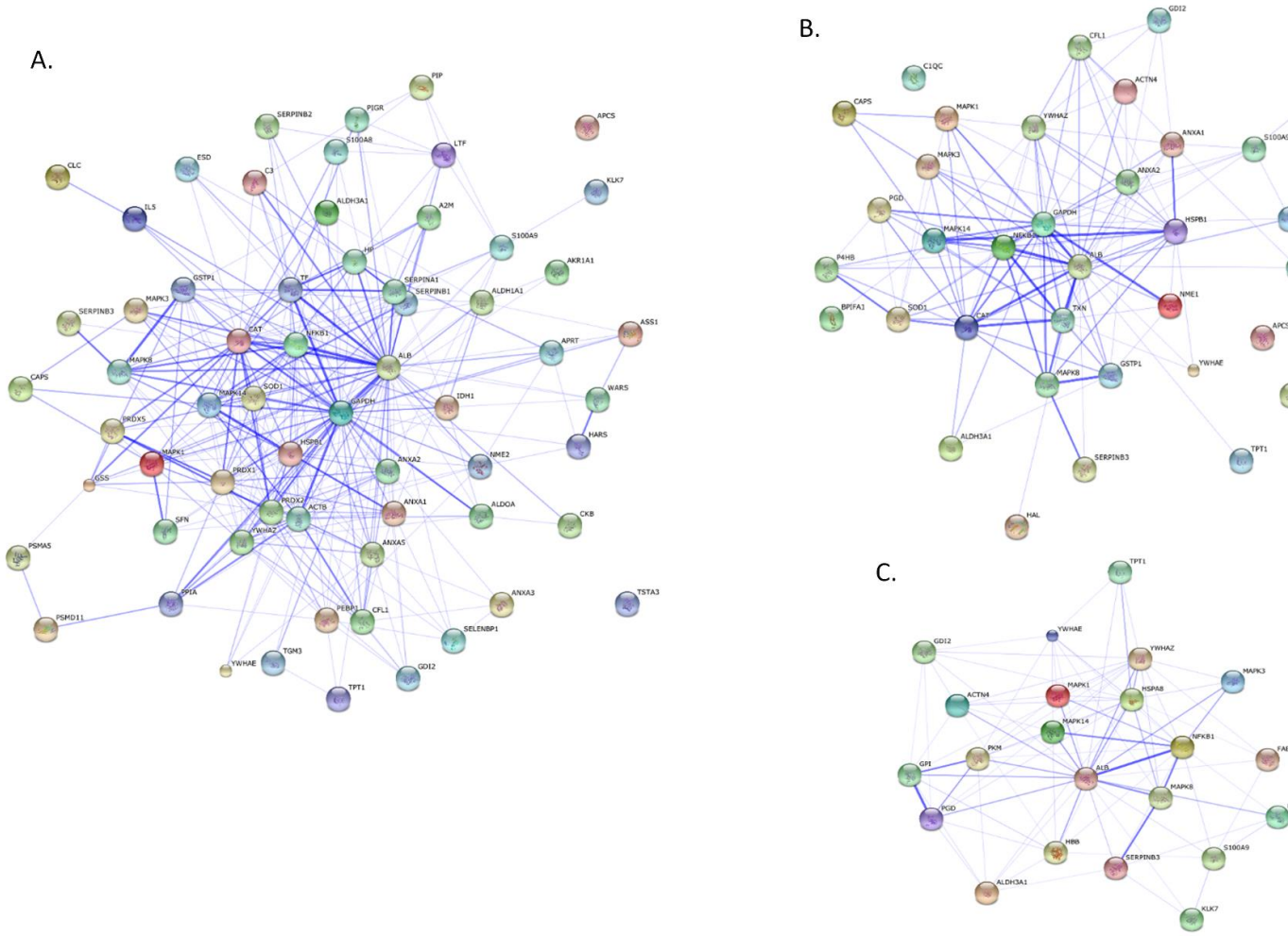
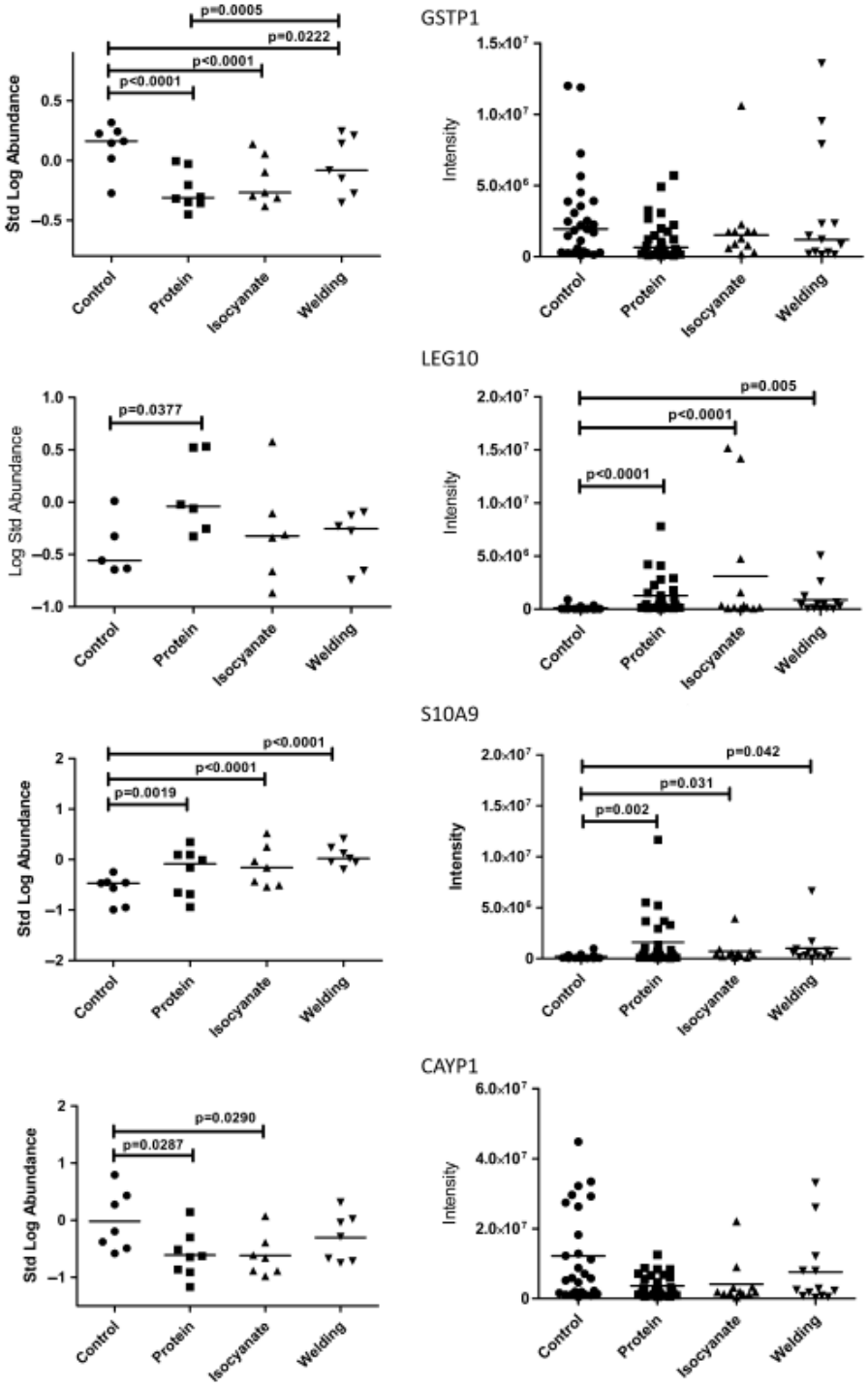


Figure S3 Protein association networks of the work-related asthma groups. Blue lines are confidence-based associations from the Strin database for the identified proteins (Table 2) in the protein allergen (A), isocyanate (B) and welding (C) groups. Stronger associations ar presented as thicker lines. Minimum required interaction score was 0,4 and all available STRING data sources were applied in the searc Mitogen-activated protein kinases (MAPK 1, 3, 8, 14), Nuclear factor kappa B (NFkB) and Interleukin-5 (IL-5) are included in the networ as they came up in individual protein network searches of the identified proteins and in IPA network searches (data not shown).


GSTP1
LEG10
S10A9
CAYP1
Std Log Abundance
Log Std Abundance
Intensity
Control
Protein
Isocyanate
Welding
p=0.0005
p=0.0222
p<0.0001
p<0.0001
p=0.0377
p=0.005
p<0.0001
p<0.0001
p<0.0001
p<0.0001
p=0.0019
p=0.042
p=0.031
p=0.002
p=0.0290
p=0.0287